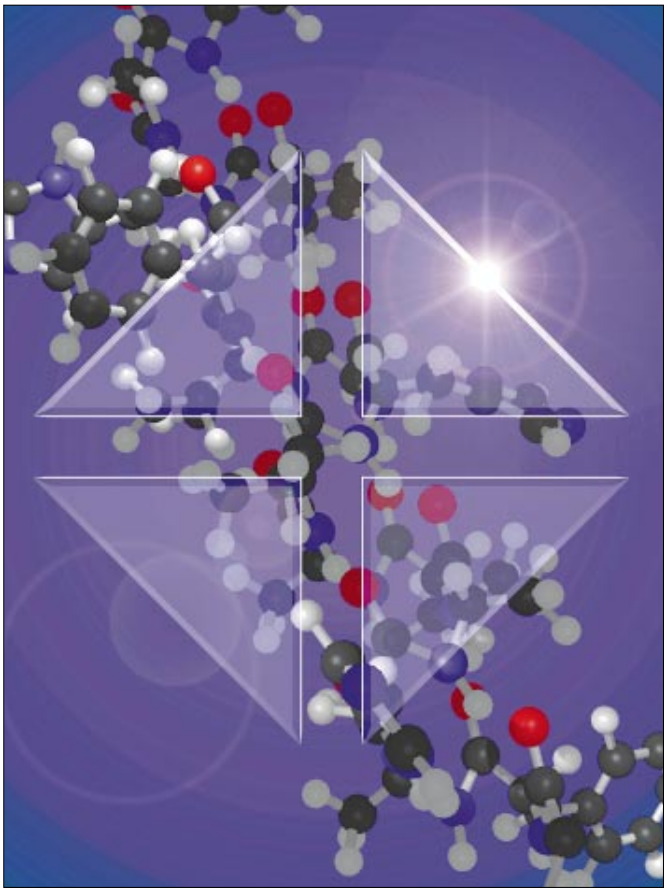
UNICORN®
version 3.00



User Manual

18–1128–28
Edition AA

Amersham Biosciences

Important user information

Reading this entire manual is recommended for full understanding of the use of this product.

The exclamation mark within an equilateral triangle is intended to alert the user to the presence of important operating and maintenance instructions in the literature accompanying the instrument.

Should you have any comments on this manual, we will be pleased to receive them at:

Amersham Biosciences AB
SE-751 84 Uppsala
Sweden

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Preface

About this manual

This manual provides a full reference to UNICORN® version 3.00 from Amersham Biosciences AB.
UNICORN is a complete package for control and supervision of chromatography systems, suitable for use with Amersham Biosciences' systems for the ÄKTA® design platform (ÄKTA® design systems), BioProcess™ System and BioPilot® System. UNICORN consists of software which runs on an IBM-compatible PC under Microsoft Windows NT 4.00*, and hardware for interfacing the controlling PC to the chromatography liquid handling module.

The manual is organised in 15 chapters and 6 appendices:

Introductory material	1. Introduction 2. UNICORN concepts 3. Logon and file handling
Methods and runs	4. Creating methods from method templates 5. Creating and editing methods 6. Performing a run 7. Scouting 8. MethodQueues
Evaluation	9. Presenting results 10. Evaluating results
System management	11. Security features 12. Network setup 13. Installation 14. Administration 15. System settings
Appendices	A. Technical specifications B. Strategy for ÄKTAdesign systems C. Strategies for UNICORN controller sytem D. Evaluation functions and instructions E. Feedback tuning F. File organisation

*Microsoft Windows NT and other Microsoft products mentioned in this User Manual are registered trademarks of the Microsoft® Corporation.

The system management section (Chapters 11-15) is intended for the system administrator and does not normally have to be read by ordinary users.

For the purposes of this user manual, examples are displayed for an ÄKTAexplorer 100 system. You should refer to the user instructions specific to the chromatgoraphy system that you are using.

Assumptions

Two broad assumptions are made in this manual:

1. You should be familiar with the chromatography systems in your installation. Refer to the appropriate System Manuals for details.

2. You should be familiar with the general principles of using Microsoft Windows NT version 4.00 or higher on your PC. Although UNICORN is a self-contained program package and does not require any direct interaction by the user with Windows NT, the user interface principles follow the conventions set by Windows NT programs.

   Many of the menu commands in UNICORN can be activated using the toolbar buttons, keyboard shortcuts and the right mouse button menu. The availability of these command options is dependent on the active field or window in which you are currently working. The function of a toolbar button is displayed when you place the mouse pointer over a button. Right mouse button menu commands are quickly found through use of the program.

Typographical conventions

Menu commands, the names of dialogue boxes and windows, the contents of dialogue boxes windows, and option buttons are written with a **bold helvetica** typeface. Menu commands are written in the order of the menu name and then the command, separated by a colon. For example:

“Select **File:Save As** to display the **Save As** dialogue. Locate the destination drive and folder and enter a file name. Click on **Save**.”

This directs you to click on the **File** menu and select the command, **Save As**. A dialogue called **Save As** is displayed in which you must locate the destination folder for the saved file and give the file a name. You then click on the button called **Save** in the dialogue to execute the save command.

A `typewriter-like` typeface is used for instructions as they appear in the text editor for methods and evaluation procedures. These are normally entered automatically by UNICORN® .

Some menu commands also have shortcut keys on the keyboard, which are written within < > marks.

Contents

Introductory material

1 Introduction

2 UNICORN concepts

2.1 UNICORN control software **2-1**

2.1.1 Strategies *2-1*
2.1.2 Stand-alone and network installations *2-1*

2.2 UNICORN user interface **2-2**

2.2.1 Toolbar Guide *2-2*
2.2.2 Software modules *2-3*
2.2.3 On-line help *2-3*
2.2.4 On-line Adviser *2-4*

2.3 Files and folders **2-4**

2.3.1 Method files *2-4*
2.3.2 Result files *2-4*

2.4 Methods **2-4**

2.4.1 Method structure *2-5*
2.4.2 Method templates *2-7*
2.4.3 BufferPrep *2-8*

2.5 Scouting **2-8**

2.6 System control **2-9**

2.6.1 Control facilities *2-9*
2.6.2 System connections *2-10*

2.7 Evaluation **2-10**

2.8 Network considerations **2-11**

2.9 Security **2-13**

3 Logon and file handling

3.1 Logging on **3-1**

3.2 Toolbar Guide **3-2**

3.3 UNICORN Main menu windows **3-4**

3.3.1 Creating a new folder *3-4*
3.3.2 Opening and running files *3-4*
3.3.3 Presenting files *3-5*
3.3.4 Finding files *3-7*
3.3.5 Copying and moving files and folders *3-9*
3.3.6 Deleting files *3-12*
3.3.7 Renaming files *3-12*
3.3.8 Backup security *3-13*

3.4 Printer setup **3-13**

3.5 Logging off **3-13**

3.6 Quitting UNICORN **3-13**

Methods and runs

4 Creating methods from method templates

4.1 Creating a new method **4-1**

4.2 Editing method variables **4-4**

4.3 Method notes **4-6**

4.4 Saving the method **4-7**

4.5 Starting a run **4-8**

4.6 Editing text instructions **4-10**

5 Creating and editing methods

5.1 Creating a new method **5-2**

5.2 Method editor interface **5-4**

5.2.1 Method editor modes *5-4*
5.2.2 Text instruction editor *5-5*
5.2.3 Run setup *5-6*

5.3 Method blocks **5-7**

5.3.1 Viewing blocks *5-7*
5.3.2 Calling blocks *5-8*
5.3.3 Adding blocks *5-9*
5.3.4 Deleting blocks *5-11*
5.3.5 Renaming blocks *5-12*
5.3.6 Importing and copying blocks *5-13*

5.4 Method instructions **5-14**

5.4.1 Viewing instructions *5-14*
5.4.2 Adding instructions *5-16*
5.4.3 Deleting instructions *5-17*
5.4.4 Changing instructions *5-18*
5.4.5 Using the gradient window *5-20*
5.4.6 The flow scheme window *5-21*

5.5 Method variables **5-22**

5.5.1 Identifying variables *5-22*
5.5.2 Defining variables *5-23*
5.5.3 Removing a variable *5-24*
5.5.4 Renaming a variable *5-24*

5.6 Run setup **5-25**

5.6.1 Variables *5-25*
5.6.2 Scouting *5-26*
5.6.3 Questions *5-28*
5.6.4 Gradient *5-31*
5.6.5 Notes *5-33*
5.6.6 Evaluation procedures *5-34*
5.6.7 Reference curves *5-37*
5.6.8 Columns *5-39*
5.6.9 BufferPrep *5-40*
5.6.10 Method Information *5-42*
5.6.11 Result Name *5-43*
5.6.12 Start protocol *5-44*

5.7 Saving the method **5-48**

5.7.1 Saving a method *5-48*
5.7.2 Saving as a template *5-49*
5.7.3 Deleting a template *5-49*

5.8 How to use selected method instructions **5-49**

5.8.1 Base instruction *5-49*
5.8.2 Instructions at the same breakpoint *5-52*

5.8.3 Controlling block and method length *5-52*
5.8.4 Messages *5-53*
5.8.5 Pausing a method *5-54*
5.8.6 Linear flow rates *5-55*
5.8.7 Eluent concentrations and gradients *5-55*
5.8.8 Conditional instructions *5-58*

5.9 The Column list **5-66**

5.9.1 Adding a new column *5-67*
5.9.2 Editing column parameters *5-71*
5.9.3 Deleting a column *5-71*
5.9.4 Selecting or changing the column in a method *5-71*

5.10 Creating and editing BufferPrep recipes **5-72**

5.10.1 Creating a BufferPrep recipe *5-72*
5.10.2 Editing a BufferPrep recipe *5-76*

6 Performing a run

6.1 Starting a method **6-2**

6.1.1 Starting from the Main menu *6-2*
6.1.2 Starting from System control *6-2*
6.1.3 Starting an Instant Run *6-3*
6.1.4 Start protocol *6-3*

6.2 Monitoring a run **6-5**

6.2.1 General window techniques *6-6*
6.2.2 Run data *6-6*
6.2.3 Curves *6-8*
6.2.4 Flow scheme *6-12*
6.2.5 Logbook *6-13*

6.3 Manual control **6-13**

6.3.1 The toolbar *6-13*
6.3.2 Manual instructions *6-15*
6.3.3 Alarms and warnings *6-16*

6.4 If communication fails **6-17**

6.5 Managing system connections **6-17**

6.5.1 Establishing a connection *6-18*
6.5.2 Connection modes *6-18*
6.5.3 Leaving and locking a system *6-20*
6.5.4 Disconnecting a system *6-20*
6.5.5 Network considerations *6-21*

6.6 Calibrating monitors **6-22**

6.6.1 Calibrating monitors for ÄKTAdesign systems *6-23*
6.6.2 Calibrating monitors for BioPilot *6-27*
6.6.3 Calibrating monitors for the BioProcess System *6-28*

6.7 Feedback tuning **6-28**

7 Scouting

7.1 Setting up scouting **7-1**

7.1.1 Scouting variables *7-4*
7.1.2 Scheme set-up *7-4*
7.1.3 Start protocol settings *7-4*

7.2 Running scouting **7-5**

7.2.1 Changing scouting settings during a run *7-5*

7.3 Scouting results **7-6**

7.3.1 Viewing results *7-6*
7.3.2 Comparing runs *7-6*
7.3.3 Printing results *7-6*

7.4 Scouting examples **7-7**

7.4.1 Scouting sample volume *7-7*
7.4.2 Screening different columns *7-7*
7.4.3 Scouting continuous gradients *7-7*
7.4.4 pH scouting *7-8*

8 MethodQueues

8.1 Setting up a MethodQueue **8-1**

8.1.1 Defining a MethodQueue *8-1*
8.1.2 MethodQueue folders and icons *8-4*

8.2 Editing MethodQueues **8-4**

8.3 Running a MethodQueue **8-5**

8.3.1 Method execution in MethodQueues *8-5*

8.4 Displaying MethodQueues **8-6**

Evaluation

9 Presenting results

9.1 Opening a result file **9-1**

9.1.1 Chromatogram *9-2*
9.1.2 Temporary chromatogram *9-2*

9.2 Basic presentation of chromatograms **9-2**

9.2.1 The chromatogram window *9-2*
9.2.2 Opening the Chromatogram Layout dialogue *9-3*
9.2.3 Choosing the curve(s) you want to see *9-5*
9.2.4 Changing curve names *9-5*
9.2.5 Changing the colour and style of curves *9-6*
9.2.6 Changing and fixing the axes *9-7*
9.2.7 Viewing information about the run *9-9*
9.2.8 Saving and applying a layout *9-10*
9.2.9 Viewing a grid in the chromatogram window *9-10*

9.3 Other presentation possibilities **9-11**

9.3.1 Showing part of a curve *9-11*
9.3.2 Reducing noise and removing ghost peaks *9-13*
9.3.3 Subtracting a blank run curve *9-15*
9.3.4 Adding curves *9-17*
9.3.5 Entering text in the chromatogram *9-17*
9.3.6 Pooling fractions *9-18*
9.3.7 Renaming chromatograms, curves and peak tables *9-19*

9.4 Comparing different runs **9-19**

9.4.1 Comparing chromatograms from different runs *9-19*
9.4.2 Comparing curves *9-22*
9.4.3 Stacking and stretching curves *9-27*
9.4.4 Mirror images of curves *9-31*
9.4.5 Matching protein activity to a curve *9-32*

9.5 Saving results **9-32**

9.6 Printing active chromatograms **9-32**

9.7 Printing reports **9-33**

9.7.1 Saving a report format *9-36*
9.7.2 Selecting a format *9-36*

9.8 Run documentation **9-37**

9.9 Exiting Evaluation **9-40**

10 Evaluating results

10.1 Integrating peaks **10-1**

10.1.1 Baseline calculation for integration *10-1*
10.1.2 Performing a basic integration *10-2*
10.1.3 Optimising peak integration *10-4*
10.1.4 Optimising the baseline parameters *10-6*
10.1.5 Manually editing a baseline *10-13*
10.1.6 Adjusting the peak limits *10-15*
10.1.7 Measuring retention time and peak heights *10-18*
10.1.8 Measuring HETP *10-19*
10.1.9 Measuring peak asymmetry *10-20*
10.1.10 Measuring resolution *10-20*

10.2 Other evaluations **10-21**

10.2.1 Peak purity and peak identification *10-21*
10.2.2 Finding the slope values for Peak Fractionation or Watch instructions *10-23*
10.2.3 Creating a curve *10-25*
10.2.4 Measuring salt concentrations in the fractions *10-27*

10.3 Automated evaluation procedures **10-28**

10.3.1 Recording a procedure *10-28*
10.3.2 Editing an existing procedure *10-30*
10.3.3 Renaming and removing procedures *10-32*
10.3.4 Points to watch *10-32*
10.3.5 Running evaluation procedures *10-33*
10.3.6 Batch runs *10-33*
10.3.7 Evaluation procedures and reports *10-35*
10.3.8 Placing a procedure on the menu and running *10-35*
10.3.9 Exporting data or curves *10-36*
10.3.10 Exporting results *10-36*
10.3.11 Copying results to the clipboard *10-37*

System management

11 Security features

11.1 Access security **11-1**

11.2 Connection security **11-1**

11.3 Data security **11-2**

11.3.1 Network communication failure *11-2*
11.3.2 Local station failure *11-3*

11.4 Security recommendations for control stations **11-3**

12 Network setup

12.1 Introduction **12-1**

12.2 Requirements **12-3**

12.3 Installation guide **12-3**

12.3.1 TCP/IP - NT domain *12-3*
12.3.2 IPX/SPX - Novell server *12-8*

13 Installation

13.1 Installation summary **13-1**

13.2 Migrating from UNICORN 2.30 to UNICORN 3.00 **13-1**

13.2.1 Before migration *13-1*
13.2.2 Migration and post-installation setup *13-2*

13.3 System requirements **13-2**

13.4 Hardware installation **13-2**

13.5 Software installation **13-6**

13.5.1 Installing UNICORN for the first time *13-6*
13.5.2 Installing selected software components after the initial installation *13-19*

14 Administration

14.1 System definitions **14-2**

14.1.1 Defining new systems *14-2*
14.1.2 Editing system definitions *14-3*

14.1.3 Deleting system definitions *14-3*

14.2 Access levels **14-4**

14.2.1 Defining access levels *14-4*
14.2.2 Access level examples *14-6*

14.3 User administration **14-9**

14.3.1 Defining new users *14-10*
14.3.2 Changing user passwords *14-11*
14.3.3 Viewing and changing user definitions *14-12*
14.3.4 Deleting users *14-12*
14.3.5 Defining new home folders *14-12*
14.3.6 Deleting home folders *14-12*

14.4 Audit trails **14-13**

14.4.1 Examining audit trails *14-13*
14.4.2 Renewing audit trail files *14-16*
14.4.3 Backing up audit trail files *14-16*

14.5 Report Generator Wizard **14-17**

14.5.1 Generating a report from the main menu *14-18*
14.5.2 Generating a report from System Control *14-22*

15 System settings

15.1 Alarms **15-2**

15.2 Specials **15-4**

15.3 Monitors **15-5**

15.4 Curves **15-7**

Appendices

A Technical specifications

A.1 System requirements **A-1**

A.1.1 Hardware requirements *A-1*
A.1.2 Software requirements *A-1*
A.1.3 Network requirements *A-2*

A.2 Control capacity **A-2**

A.2.1 Stand-alone installations *A-2*
A.2.2 Network installations *A-2*

A.3 Data sampling **A-2**

B Strategy for ÄKTAexplorer 100

B.1 Method instructions **B-1**

B.1.1 Pump *B-1*
B.1.2 Flowpath *B-3*
B.1.3 Alarms&Monitors *B-6*
B.1.4 Watch *B-7*
B.1.5 Other *B-8*

B.2 Manual control **B-9**

B.2.1 Pump *B-9*
B.2.2 Flowpath *B-9*
B.2.3 Alarms&Monitors *B-9*
B.2.4 Other *B-10*

B.3 System settings instructions **B-10**

B.3.1 Alarms *B-10*
B.3.2 Specials *B-11*
B.3.3 Monitors *B-12*
B.3.4 Curve *B-14*

C Systems using the UNICORN controller box

C.1 Method instructions **C-1**

C.1.1 Pump *C-1*
C.1.2 Flowpath *C-2*
C.1.3 Alarms&Monitors *C-5*

C.1.4 Watch *C-6*
C.1.5 Other *C-7*

C.2 Manual control instructions **C-8**

C.2.1 Pump *C-8*
C.2.2 Flowpath *C-9*
C.2.3 Alarms&Mon *C-9*
C.2.4 Other *C-9*

C.3 System settings instructions **C-10**

C.3.1 Alarms *C-10*
C.3.2 Specials *C-11*
C.3.3 Monitors *C-12*
C.3.4 Curves *C-12*

D Evaluation functions and instructions

D.1 Smoothing algorithms **D-1**

D.1.1 Moving Average *D-1*
D.1.2 Autoregressive *D-1*
D.1.3 Median *D-2*

D.2 Baseline calculation theory **D-2**

D.2.1 Finding baseline segments *D-3*
D.2.2 Selecting baseline points *D-5*
D.2.3 Drawing the baseline *D-5*
D.2.4 Estimating the baseline parameters from the source curve *D-5*
D.2.5 Measuring the Slope limit using Differentiate and curve co-ordinates *D-6*

D.3 Peak table column components **D-6**

D.4 Evaluation procedure **D-10**

D.4.1 Curve operations *D-10*
D.4.2 Integration *D-13*
D.4.3 File Operations *D-14*
D.4.4 Export *D-15*
D.4.5 Chromatogram functions *D-17*
D.4.6 Other *D-18*

E Feedback tuning

E.1 Flow rate tuning **E-2**

E.2 Gradient tuning **E-3**

F File organisation

F.1 Stand-alone installations **F-1**

F.2 Network installations **F-1**

F.2.1 Local and remote computers *F-1*

F.2.2 Network server *F-1*

Introductory material

Methods and runs

Evaluation

System management

Appendices

1 Introduction

UNICORN® is a control system developed and marketed by Amersham Biosciences AB for real-time control of chromatographic separation systems from a personal computer. The package operates together with systems in the ÄKTA® design platform (referred to as ÄKTAdesign systems), BioPilot® System and BioProcess® System from Amersham Biosciences. Standard configurations are provided for ÄKTAdesign systems and BioPilot System, while configurations for process-scale separation with BioProcess System may be customised for a particular installation. UNICORN runs under the operating system, Microsoft Windows NT version 4.0 or higher.

Functional features of UNICORN 3.00 include:

- One PC may control up to 4 separation systems directly.
- Network support allows any number of systems to be run from one PC.
- Method templates, providing method frameworks for most common applications, eliminating the need to program methods from scratch.
- Modular method definition in the method templates, reflecting the separate steps in a chromatographic process (e.g. equilibration, sample loading, elution, column regeneration.
- Scouting facilities, allowing any parameter to be varied automatically in repeated runs to facilitate process development and optimisation.
- Dynamic graphical overview of active runs.
- User-definable alarm and warning limits for monitor signals.
- Programmed sequential operation of multi-step separations.
- Batch operation and process documentation in accordance with the requirements of Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP).
- Comprehensive data evaluation software.

In addition, UNICORN offers a comprehensive security system:

- Password control for all users, with access authorisation for other users' method and result files.
- Customised definition of access control levels.
- Audit trail for system operation.

Note: UNICORN must be correctly installed for stand-alone or network operation before the software can be used. Network considerations, software installation and administration of system and user definitions are described in Chapters 12, 13 and 14.

An optional Analysis module is separately available for UNICORN 3.00, which enables quantitation and molecular size determinations. The Analysis module is described in its own user manual.

2 UNICORN concepts

This chapter introduces the basic concepts that are specific to UNICORN. For a description of how to work with the Windows NT operating system, see your Windows NT system documentation.

Material in this chapter is divided into 8 sections, dealing with:

- UNICORN control software
- UNICORN user interface
- Files and folders
- Methods and method structure
- System control
- Scouting
- Evaluation
- Network considerations
- Security and administration

2.1 UNICORN control software

UNICORN runs under the Windows NT operating system, and provides facilities for method-controlled operation of chromatography systems as well as real-time monitoring and subsequent evaluation of the separation process.

2.1.1 Strategies

Part of UNICORN software (referred to as the strategy) is system specific. The strategy defines what is available in method and manual instructions, system settings, run data, curves and method templates. Most of this manual describes the user interface in UNICORN independent of the strategy. Strategy-dependent instructions are listed in Appendix B and C.

2.1.2 Stand-alone and network installations

UNICORN may be installed either on a stand-alone computer or in a network.

- In a stand-alone installation, up to four chromatography systems may be physically connected to and controlled from the computer where UNICORN is installed.

- In a network installation, each chromatography system is physically connected to one computer in the network, but may be controlled from any computer in the network on which the software is installed. A computer to which chromatography systems are physically connected is referred to as a local station. Other computers in a network installation are called remote stations.

Note: It is not necessary to install UNICORN for network control if you only want to be able to save method and result files on a server disk. Just define a home folder on the server disk (see Section 14.3.1) if the computer itself is connected to a network. Also, accessing network printers does not require the UNICORN network installation. You can install a local stations and connect to a network printer with standard Windows NT network functionality.

2.2 UNICORN user interface

2.2.1 Toolbar Guide

The UNICORN Toolbar Guide dialogue is shown after start-up and logon reminding you about the Main menu toolbar buttons.

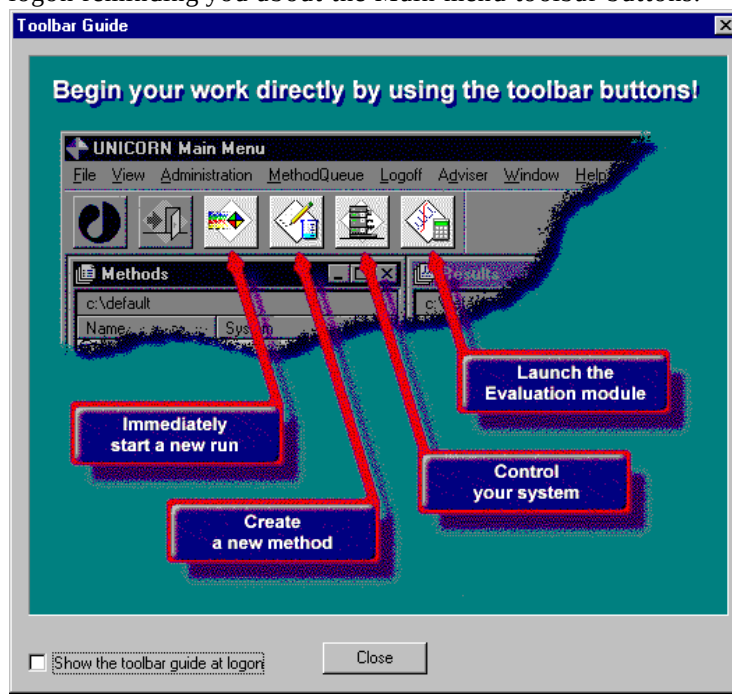


Figure 2-1. UNICORN Toolbar Guide dialogue.

The Main menu toolbar buttons allow you to begin using UNICORN quickly, for example, to create a new method in the Method editor, start an instant run, open a result file for evaluation, or execute manual instructions in System control (see Section 3.2).

2.2.2 Software modules

UNICORN control software consists of four integrated modules:

- The **Main menu**, with functions for file handling and administrative routines such as definition of available chromatography systems and maintenance of user profiles.
- The **Method editor**, where methods for pre-programmed control of chromatography systems are created and edited.
- The **System control** module, which permits manual or method-based control of chromatography systems and on-line monitoring of separation processes. There may be up to four independent system control modules on one computer, for controlling up to four separate systems.
- The **Evaluation module**, with extensive facilities for presenting and evaluating stored results from separation processes.

These modules are present on the Windows NT taskbar.

To minimize a module to the taskbar, click on the Minimize button at the right-hand end of the window title bar. To minimize the whole of UNICORN click on the <Windows + M> keys on the keyboard.

Note: Minimizing a module window to the taskbar does not close the module. Once opened, UNICORN modules remain active until you quit the program. A minimised System control module may thus be actively in control of a running process.

2.2.3 On-line help

A comprehensive on-line help utility is included in UNICORN software. Entry to the general help utility can be accessed from the **Help** menu. Dialogue- or window-specific help topics can be obtained by clicking on the **Help** button in the dialogue or by pressing <F1> on the keyboard. In the dialogues for method instructions, procedure instructions and system settings, pressing F1 when an instruction is highlighted will display an information box with short help on the function and use of the selected instruction.

2.2.4 On-line Adviser

There is an on-line Adviser for ÄKTAdesign systems giving you detailed information about different media and methods able to be used, in addition to system information and buffers.

2.3 Files and folders

UNICORN Main menu interface divides user files into two categories, for methods and results (see Figure 2-1). Only folders to which the current user has access are shown in the Main menu windows, **Method** window and **Results** window. Files may be displayed in several viewing options (see Chapter 3 for more details).

2.3.1 Method files

Method files contain instructions for controlling a run and are shown in the **Methods** window of the Main menu.

The **Methods** window also displays icons for MethodQueues, which allow several methods to be run in an automatic pre-programmed sequence on the same or different systems.

2.3.2 Result files

Result files are created by UNICORN when a method is run and contain:

- A copy of the method used in the run.
- Run data from the monitors in the chromatography system (e.g. UV absorbance, flow rate, conductivity etc.).
- Saved results from evaluation of the run data (see Chapter 10).
- Run documentation including information on, for example, the run log, calibration settings, scouting parameters, text method etc.

2.4 Methods

Chromatography runs are programmed as *methods* in UNICORN. This section gives a brief overview of the concepts and principles of methods. See Chapters 4 and 5 for a description of how to program methods, and Chapter 6 for how methods are used to control chromatography systems.

2.4.1 Method structure

Blocks

Methods in UNICORN are usually divided into blocks. Blocks typically represent well-defined steps in the separation procedure, such as:

- column equilibration
- sample application
- elution
- column wash

Blocks are also used for operations which are to be performed conditionally (e.g. starting fraction collection if a UV-absorbance peak is detected). The user determines the number of blocks in a method, as well as the names and functions of the blocks.

Method base

Method blocks are written in one of three bases, which defines the unit for the breakpoints in the block:

- time (min)
- volume (ml or l according to the strategy)
- column volume (set by the user)

Different blocks in the same method may be written on different bases: for example a column wash block might be written in terms of column volumes while sample loading might be more appropriate in absolute volume.

Instructions

Each block in a method consists of a series of instructions (see Fig. 2-3) which request specific operations in the system. For example:

```
Flow 8.0 {ml/min}
```

sets flow rate for the pump to 8.0 ml/min.

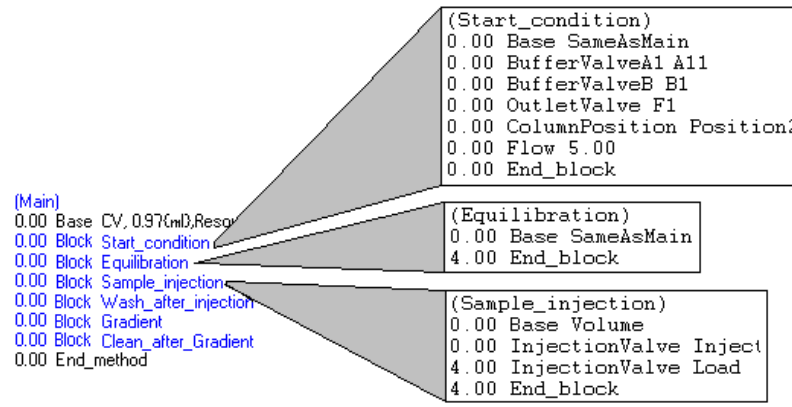


Figure 2-2. Relationship between blocks and instructions. The method (left) is written as a series of blocks, each of which consists of instructions for performing one or more specified tasks (right).

Breakpoints

Each instruction in a method block is issued at a specified breakpoint according to the method base. The first instruction in a block is always at breakpoint 0, and all other breakpoints are counted from this point. For example, the instruction

```
5.00 Flow   8.0
```

will set the flow rate to 8.0 ml/min at 5 units (minutes, ml or column volumes according to the method base) after the start of the block.

Method variables

Breakpoint values and instruction parameters may be defined as variables. This is a powerful facility for:

- constructing a "framework" method with default parameter values which may easily be changed either to create variants of the same method or to adjust the parameter values at the start of a run (see Section 4.5).
- constructing a method for use in automatic method scouting, where one or a few parameters are varied systematically (see below and Chapter 7).

Using variables makes it easy to adapt a method to a particular chromatography run. For example, in the block below, the breakpoint for switching the InjectionValve from Inject to Load is defined as a variable with the name `Empty_loop_with`. The value supplied for

this variable when the method is run will set the volume with which the loop is emptied:

```
(Sample_Injection)
0.00  Base Volume
0.00  InjectionValve Inject
(4.00)#Empty_loop_with InjectionValve Load
4.00  End_Block
```

Variable names in method instructions are always preceded by the # character. The value for the variable is shown in parentheses in the instruction (in the example above, `4.00` is the value for the variable `Empty_loop_with`).

By using variables, a method may be displayed either in detail as Text instructions or in a condensed form as variable values in Run setup mode. This is illustrated in Figure 2-4. The Run setup mode is displayed when the method is run, allowing variable values to be set at the beginning of the run. Up to 64 variables can be included in a single method.

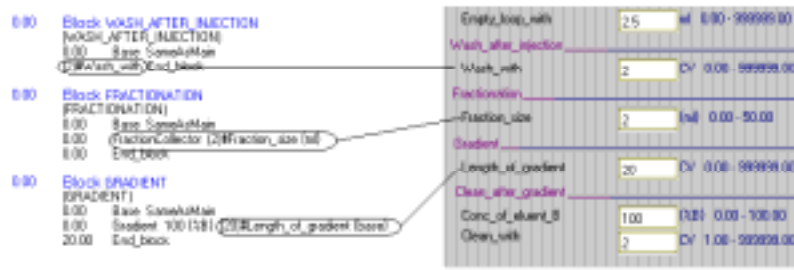


Figure 2-3. Relationship between variables in Text instructions and in the Variables page of run set-up.

2.4.2 Method templates

Method templates are "skeleton" methods which provide convenient starting points for developing customised methods (see Chapter 4 for more details).

Method templates for all chromatography techniques are supplied with UNICORN installations for ÄKTAdesign systems and BioPilot. New methods are created by selecting a suitable technique, template and column. By selecting a column, recommended flow rate, column volume and pressure limit for the selected column are copied into the method. The method can then, if necessary, be modified on the Variables page or in the Text instructions. Fully adequate customised methods for many applications can be created simply by adjusting the values of method variables in a suitable template.

2.4.3 BufferPrep

BufferPrep is only available for some ÄKTAdesign systems. BufferPrep allows a buffer of any pH and salt concentration to be prepared on-line from four stock solutions. BufferPrep should only be used for ion exchange chromatography.

2.5 Scouting

Scouting is a powerful facility for repeating a method run automatically with predetermined changes in values for one or more variables in the method. Typically, scouting is used for optimising chromatographic processes or monitoring column performance, e.g. by determining the dependence of resolution on flow rate or gradient slope.

A scouting scheme in UNICORN is defined as part of the method. Values for the scouting variables may be set in the method and/or adjusted when the method is started. The following scheme for example scouts for flow rate and sample volume optimisation:

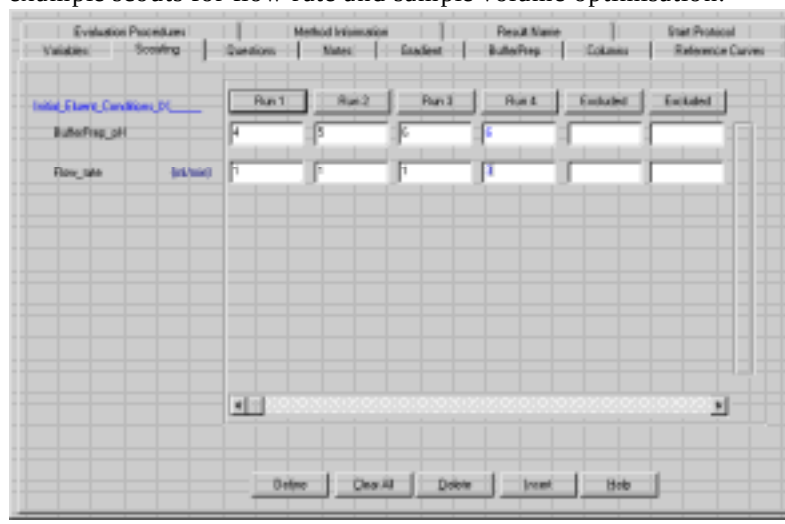


Figure 2-4. Scouting scheme for testing flow rate and sample volume parameters. Only the flow rate is varied in this example.

2.6 System control

2.6.1 Control facilities

The system control module allows independent control of up to four chromatography systems from one computer, with continuous real-time monitoring of the separation process. The run status can be displayed as:

- numerical display of run data from selected monitors
- graphical display of curves from monitors
- a flow scheme showing the current buffer flow path in the system
- a logbook recording the control events in the run.

Systems can be controlled either manually with interactive commands or through pre-programmed methods.

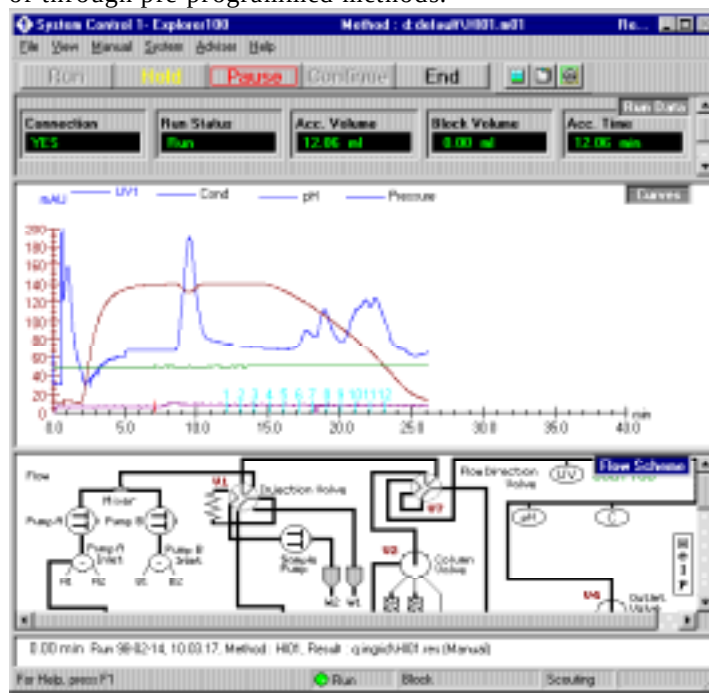


Figure 2-5. The system control screen.

By using MethodQueue facilities, several methods may be run in a pre-defined automatic sequence involving one or more chromatography systems. With suitable chromatography system equipment, this allows unattended operation of quite complex multi-step separation processes.

2.6.2 System connections

For controlling a separation process, the operator establishes a *connection* between the computer and the chromatography system in one of the system control windows on UNICORN desktop. Two kind of connections may be established:

- *Control mode connections* which permit full control of the connected system.
- *View mode connections* from which the progress of the separation can be monitored but the system cannot be controlled.

A system can be started from a computer in, for example, the laboratory. Control of the system can be released without affecting the run and the control of the system can be later taken from another computer station, for example, in the office.

Each chromatography system can have only *one* control mode connection at any one time, but it can have *several* view mode connections. In a network installation, the same or different users may establish simultaneous view mode connections to one system on different computers. This allows a running process to be monitored from several locations at the same time.

2.7 Evaluation

The evaluation module provides extensive facilities for presentation and evaluation of separation results. Essential features of evaluation include:

- *Curve manipulation*. A wide range of operations can be performed on curves, such as addition and subtraction of two curves, differentiation, integration, normalization and scaling. The original raw data curves are always kept unmodified in the result file.
- *Curve comparisons*. Curves from different result files can easily be compared in the evaluation module.
- *Peak purity and peak identification*. Ratios between UV curves measured at different wavelengths give useful information about peak purity or peak identity.
- *Peak integration*. Peak area calculations are performed with respect to a baseline either set by the operator or calculated by the software. Peak data are presented in a tabular form.
- *Fraction histograms*. If the result file contains fraction marks, a curve can be converted automatically to a histogram for collected fractions.

- *Evaluation procedures*. Operations performed in the evaluation module can be recorded as an evaluation procedure and repeated for other result files with a single menu command. Evaluation procedures may be executed either automatically on completion of a method run or interactively from within the evaluation module.
- *Reports*. Comprehensive reports of the evaluation results can be generated for hard-copy documentation of the separation process. Generation and printing of reports may be included as an operation in an evaluation procedure to automate process evaluation and documentation.

The evaluation module is described in detail in Chapters 9 and 10.

2.8 Network considerations

Figure 2-7 illustrates how a networked UNICORN installation can be organised. There are two kinds of PC where UNICORN software is installed:

- A local station is a PC to which chromatography systems are physically connected.
- A remote station is a PC to which no systems are physically connected, but which can control systems over a network link.

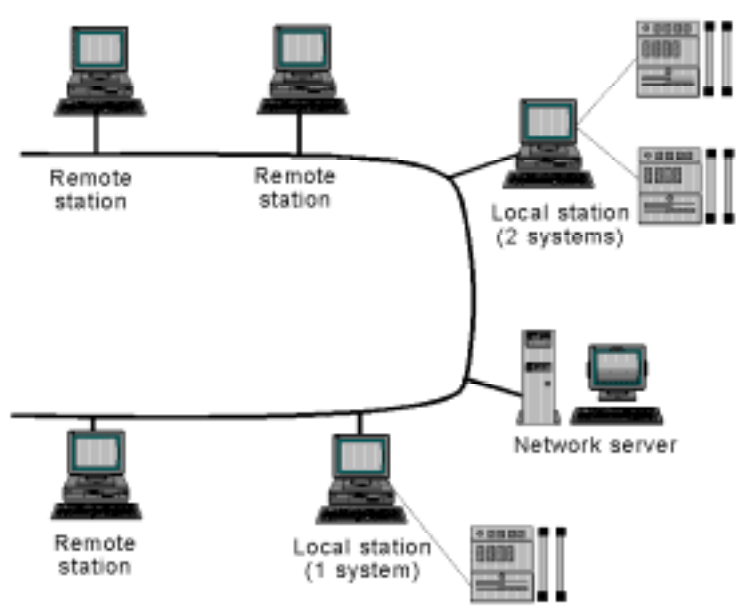


Figure 2-6. A network installation with 3 chromatography systems and 5 stations (3 remote and 2 local). The systems can be controlled from any computer except the network server.

Chromatography systems in the network can be controlled from either remote or local stations. A local station in a network can also be used as a remote station to control other systems.

Note: If a run is being controlled from a remote station and a network communication error occurs, the run will continue under the control of the local station. Results will be saved in the Failed folder on the local station (see Section 6.4). A control mode connection can be established on the local station to control the run.

For a chromatography system to be accessible in the network, the local station must be switched on and logged on to the network. UNICORN user interface does not however need to be started on the local station.

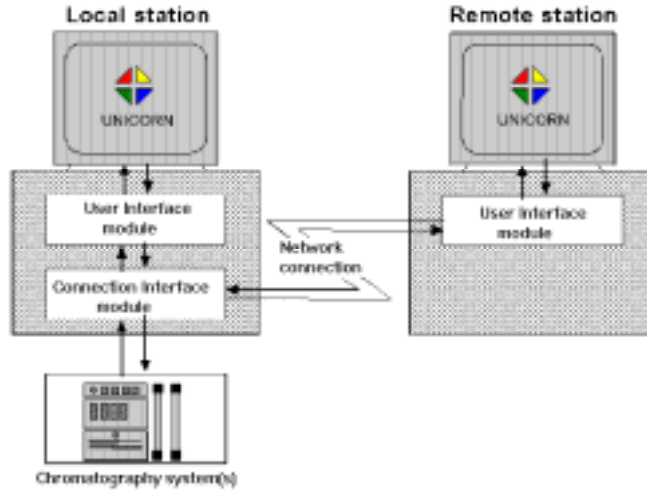


Figure 2-7. The connection interface module in a local station is started automatically and runs separately from the user interface module. This allows systems connected to the local station to be controlled from a remote station without running the user interface module on the local station.

A local station can be used to control the chromatography systems directly connected to the PC without logging on to the network. Method and result files stored on network drives will of course not be accessible. For runs performed in this stand-alone mode where the result file is directed to a network drive, the results will be saved in the Failed folder on the local station (see Section 6.4).

2.9 Security

Security features in UNICORN include:

- *Access security.* Use of UNICORN is restricted to authorised users. Each user is assigned an access level which defines the functions that the user is permitted to use.
- *Connection security.* Running systems may only be controlled from one connection. Systems may be locked with a password to prevent other users from changing run parameters.
- *Data security.* Result files can be saved automatically at pre-set intervals during a run to minimise data loss in the event of system failure. In a network installation, results are saved on the local station if network communication fails.

Security features are discussed in more detail in Chapter 11. Network and administrative aspects are discussed in Chapters 13 and 14 respectively.

3 Logon and file handling

3.1 Logging on

When you start the computer you must log on to Windows NT before you can log on to UNICORN and begin working. Logging on to Windows NT will automatically connect you up to the network if NT has been so configured. Network connection is not essential for local control of a system.

1. To start UNICORN, locate the program in the Windows NT Start button under **Programs:Unicorn:Unicorn 3.0**.



If UNICORN is already started and the previous user has logged off, click on the **Logon** menu command or click on **Logon/Logoff** button the in the Main menu module.

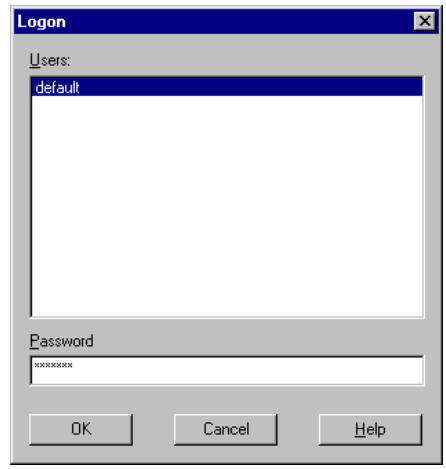


Figure 3-1. The Logon dialogue.

Click on your username in the list and type your password. Click on the **OK** button to log on. If you cannot remember your password, you cannot log on to UNICORN. Ask your system administrator or other user with sufficient authorisation to give you a new password.

Note: If UNICORN has been installed so that no password is required for logon, you need only select you username and click on **OK** to proceed.

Press the **Cancel** button to abandon the logon attempt.

Network installations

In a network installation, you must be logged on to the network before starting UNICORN. Any computer station in the network with UNICORN software installed can be used to log on to UNICORN. You can log on with the same username and password on any number of computers simultaneously.

Each chromatography system can have only *one* control mode connection at any one time, but it can have *several* view mode connections. In a network installation, the same or different users may establish simultaneous view mode connections to one system on different computers. This allows a running process to be monitored from several locations at the same time. Multiple logons with the same username are treated internally as separate users for the purpose of System control.

Note: Do not confuse Windows NT/network logon with UNICORN logon. You log on to the network to gain access to network resources (shared drives, printers and other networked equipment). You log on to UNICORN to gain access to the chromatography systems that are installed in the network. The username and password for logging on to the network are entirely independent of the those for logging on to UNICORN.

3.2 Toolbar Guide

The UNICORN Toolbar Guide dialogue is shown after start-up and logon reminding you about the Main menu toolbar buttons.

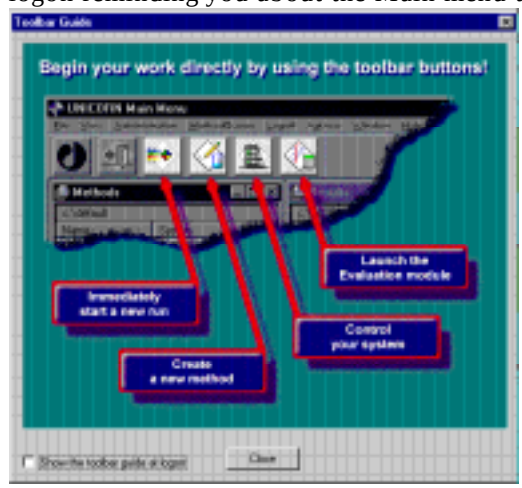


Figure 3-2. UNICORN Toolbar Guide dialogue.

The Main menu toolbar buttons allow you to begin using UNICORN quickly, for example, to create a new method in the Method editor, start an instant run, open a result file for evaluation, or execute manual instructions in System control.

The toolbar buttons are:



About UNICORN

This gives you information about the UNICORN version installed, copyright and web address for obtaining more information.



Logon/Logoff

This allows you to log on or off UNICORN as appropriate.



Instant Run

This opens the **Instant run** dialogue (Fig. 3-3) in which you can select the system to run, technique, template and column. Press on the **Run** button to view the Start protocol and to start the run (see Chapter 6).

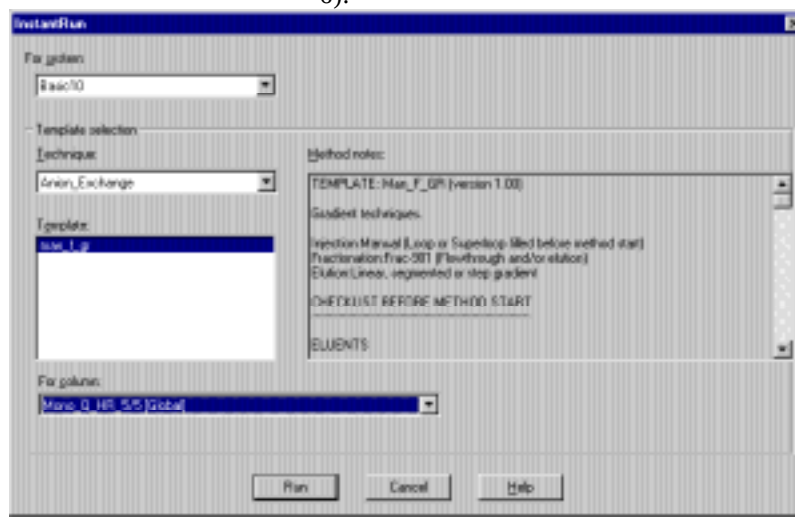


Figure 3-3. Instant Run dialogue.

Note: Use of this function requires that templates are defined. Standard systems are supplied with templates, but custom systems require that the user makes templates.



New Method

This immediately starts the Method editor module and displays the **New Method** dialogue (see Section 4.1).



System Control This activates the first connected System control and displays the **Manual instruction** dialogue (see Section 6.3.2).



Evaluation This displays the **Open result** dialogue. Select a result file and click on **OK** to launch the Evaluation module (see Chapter 9).

3.3 UNICORN Main menu windows

The two Main menu windows display the folders to which you have access within UNICORN and the method and result files within the currently open folder respectively. You can only see method files written for systems to which you have access.

3.3.1 Creating a new folder

To create a new user-specific folder:

1. Select the appropriate window, **Methods** or **Results**, in which you want to create a new folder.

2. Select **File:New:Folder** or **New Folder** from the right mouse button menu. The **Create New Folder** dialogue is displayed.

3. Enter the name of the new folder and click on **OK**. The new folder is displayed in the appropriate window. Any user that has access set to the main folder in which the new folder was created also has access to the folders and files contained therein.

3.3.2 Opening and running files

To open a method file in the Method editor or a result file in the Evaluation module, click on a file in the **Methods** window or **Results** window respectively and select **File:Open**, or click on the file with the right mouse button and select **Open** from the menu. Alternatively, double click on a file to open it.

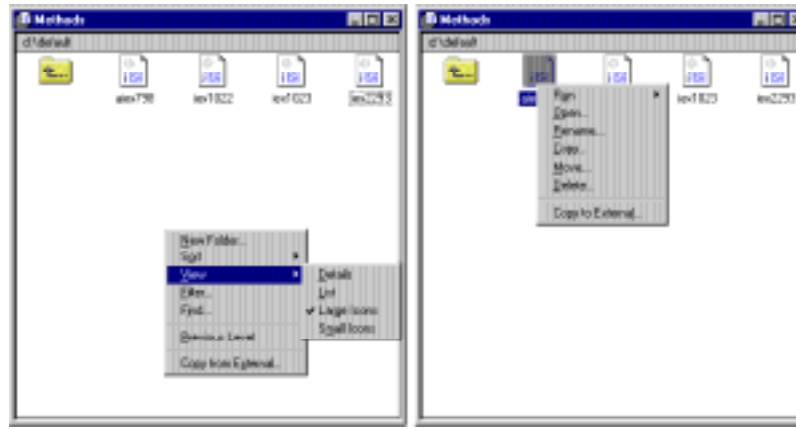


Figure 3-4. The Method window with right mouse button selected in the window (left) and the right mouse button menu for a selected icon within the window.



Method files can be run directly in the System control module. Alternatively, click on a file in the Main menu **Methods** window and select **File:Run**, or click on the file with the right mouse button and select **Run** from the menu.

3.3.3 Presenting files

The way files are presented in the windows can be set from the **File** menu or from the mouse right button menu. Presentation options are:

- view mode
- sorting order
- filter (for displaying only a chosen set of objects, e.g. methods for one system)

View mode

You can select to display the contents of the windows in several Windows NT views from the **View** menu or **View** options in the right mouse button menus. View the files either as a details list (**View:Details**), a simple list (**View:List**), large icons (**View:Large Icons**) or small icons (**View:Small Icons**).

The details list includes a small icon identifying the type of object, file name, file type, and the last modified date and time.

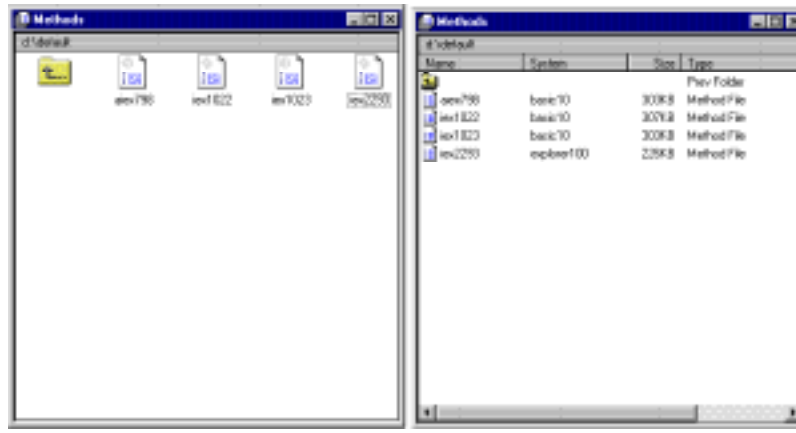


Figure 3-5. Icon and detail display modes illustrated for the Methods window.

Sorting order

In the details list viewing mode files can be sorted in the window according to one of:

Name	alphabetical order or reverse alphabetical order
System	alphabetical order or reverse alphabetical order (**Method** window only)
Size	smallest or largest files first
Type	alphabetical order of file extension type
Modified	last recently modified files first

To change the sorting order, choose **Sort** from the right mouse button menu or **File:Sort**, and choose the appropriate sorting order from the menu cascade. Alternatively, click on the column headers in the window for **Name**, **System**, **Size, Type** and **Modified** to change the file sorting accordingly. Click a second time on the same sorting option and the files are sorted in reverse alphabetical order, increasing file size etc. as appropriate to the selection. Changing the sorting order affects only the currently active window.

Filter

To restrict the files displayed according to file name or the system with which they are associated, choose **Filter** from the right mouse button menu or select **File:Filter**. Mark the system(s) for which you want to

display files, and enter a file name specification if required. Click on **OK** to activate the filter. The filter affects the display in *both* windows.

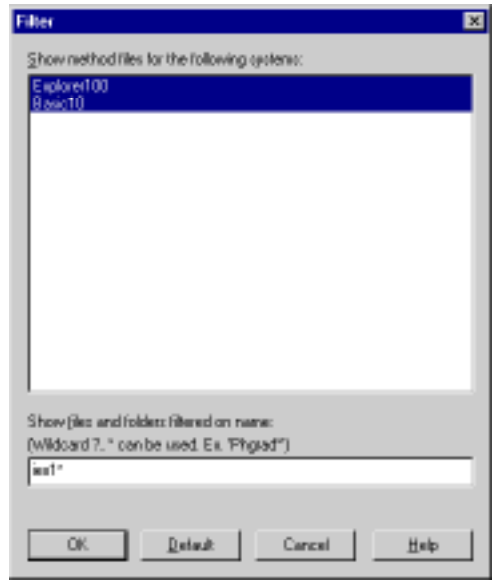


Figure 3-6. The Filter dialogue.

You can use standard Windows wildcard characters in the file name specification (* stands for any number of characters, ? for any single character). For example:

iex	will display only files named iex
iex*	will display all files with names beginning with iex
*iex	will display all files with names ending with iex
?iex	will display only 4-character names ending with iex

If a filter is active, this is indicated in the title bar of the panel (e.g. Results : filtered igf*). To display all files, choose **Filter** and click on **View All**.

3.3.4 Finding files

To find a file:

1. Choose **Find** from the right mouse button menu or select **File:Find**. In the displayed **Find file** dialogue, enter a file name specification in the **Search for files filtered on name** field.



Figure 3-7. The Find file dialogue.

You can use standard Windows wildcard characters in the file name specification (see above under Filter).

2. You can restrict the search further if required:

   - Choose file type from the pull-down menu for **Type** (**All**, **Folders**, **Method files** or **MethodQueue files** for the **Methods** window; **All**, **Folders**, **Result files** or **Scouting files** for the **Results** window).
   - Click on **Date range** and use the slide bar to set the date limits for the search. Click on **OK**.
   - Check **Search all folders** to search through all the folders to which you have access. If **Search all folders** is not checked, the search will be restricted to the current folder and sub-folders below.

3. Click on **Find** when you have entered all parameters. The result of the search is shown in the **Found files** box.

4. Double-click on a file in this list to return to the Main menu with the selected file highlighted in the appropriate window. If you click on **Close** (with or without selecting a file), you will return to the Main menu with the window display unchanged.

3.3.5 Copying and moving files and folders

You can copy and move files and folders to another folder that is specific to your user logon name. You can also copy or move files to and from an external drive and folders available on the network. If you copy or move a folder, all files within the folder will also be copied or moved.

Copying or moving files and folders

1. Select one or more files or folders in either the **Methods** or **Results** window of the Main menu. To select multiple files or folders, hold down the <Ctrl> key while you click on the file/folder names or icons.

2. Click with the right mouse button on any file/folder icon and choose the **Copy** or **Move** command or select **File:Copy** or **File:Move**. The **Copy** or **Move** dialogue is displayed respectively.

3. Select an available folder or the diskette drive to which you want to copy or move the file/folder and click on **OK**. Copied files and folders are user-specific.

Note 1: You cannot copy or move files between the **Methods** and **Results** windows of the Main menu.

Note 2: Explicit authorisation is required to copy or move files (see Section 14.2).

Note 3: To copy a file within the same folder, open the file in the relevant UNICORN module, e.g. a method file in Method editor or a result file in the Evaluation module, and use the **File:Save as** command in the module to save the file with a different name from the original.

Note 4: When copying to a diskette (a:) use **Copy to external** so that the files are automatically compressed.

Copying files to external

Copying files to external may be useful when you want to store all results, documentation etc. in a common project folder on the network, or want to back up the files in a special place.

To copy a method or result file to external:

1. Select the file to be copied in either the **Methods** window or **Results** window.

2. Select **Copy to external** from the right mouse button menu or select **File:Copy to external**. The **Copy to external** dialogue is displayed.

3. Select the destination drive and folder and click on the **Save** button.

Note: If you select the 3½" Floppy Drive (a:) as the destination drive, the files will be automatically compressed into a .zip file thus allowing approximately 5-10 times the storage capacity. Files are automatically decompressed when using the **Copy from external** operation (see below). The zip function does not work if you select the **Copy** function.

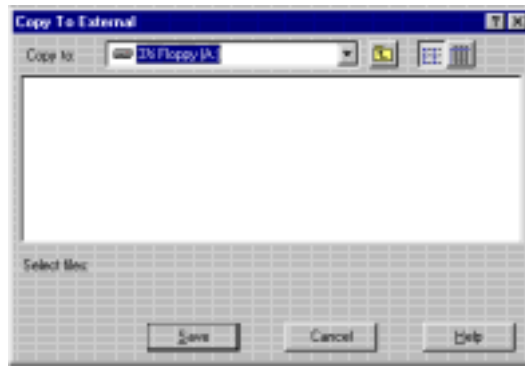


Figure 3-8. Copy to external dialogue.

Copy files from external

Method and result files can be copied from external. If the selected files have been compressed using the **Copy to external** function, then these will be automatically decompressed. To copy a method or result file from external:

1. Select the destination folder in the **Methods** window or **Results** window.

2. Without selecting a file icon, bring up the right menu button menu and select **Copy from external** or select **File:Copy from external**.

The **Copy from external** dialogue is displayed.

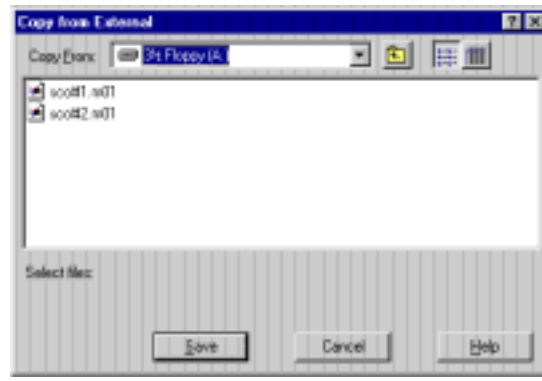


Figure 3-9. Copy from external dialogue, in this example, used to copy method files.

4. Select the wanted file(s) from the relevant source drive and folder. Click on the **Save** button.

5. If result file(s) were selected, these will be copied into the previously designated folder in the **Results** window.

6. If method file(s) were selected, the **Method-System connection** dialogue is displayed.

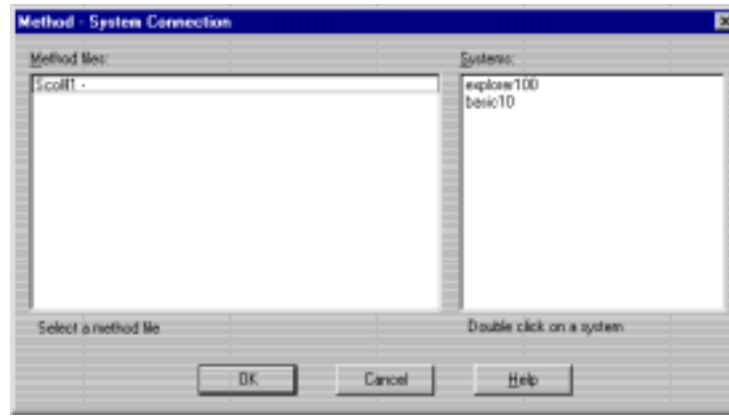


Figure 3-10. Method-System connection dialogue.

Each copied method listed in the **Method files** field must in turn be connected to the same type of system (same strategy) for which the method was originally created, listed in the **Systems** field. Highlight a method and double click on a system. Click on the **OK** button.

The **Method-System connection** dialogue is displayed again listing the remaining methods to be connected. Repeat the process until all methods have been connected.

Note: Method syntax errors may arise if a method created on one system is connected to a different type of system using the copy from external facility.

If at any time you press on the **Cancel** button, the **Method - System connection** dialogue is closed. However, it will reappear each time you perform other copy to/from external procedures for method files.

Method files that have been copied in and connected are displayed in the previously designated folder in the **Methods** window.

3.3.6 Deleting files

To delete a file or folder:

1. Select the item(s) to be deleted in the **Methods** or **Results** window of the Main menu. To select multiple files, hold down the <Ctrl> key while you click on the file names or icons.

2. Click with the right mouse button on any file icon and choose **Delete** from the menu, or choose **File:Delete**.

3. Confirm the deletion in the dialogue.

Note 1: Home folders cannot be deleted by this method (see Section 14.3).

Note 2: Explicit authorisation is required to delete files (see Section 14.2).

Note 3: A file that has been deleted cannot be recovered except by restoring a back-up copy.

3.3.7 Renaming files

To rename a file or folder:

1. Select a file or folder to be renamed in the **Methods** or **Results** window of the Main menu.

2. Click with the right mouse button on any file icon and choose **Rename** from the menu, or choose **File:Rename**. The **Rename** dialogue is displayed

3. Enter the new name for the file and click on **OK**.

3.3.8 Backup security

To protect important data against accidental deletion or loss in the event of hard disk failure, backup copies should be taken at regular intervals.

This can be best achieved by having the UNICORN folders on the server (if available) and working directly from these folders. Alternatively, you can use the **File:Copy to external** function to save files onto the network server. It is standard practice for backups to be made of the server folders. The responsibility for making backup copies rests entirely with the user. Amersham Biosciences cannot undertake to replace method programs lost as a result of computer failure or other incident.

3.4 Printer setup

UNICORN 3.0 uses the default printer and printer settings installed on your computer. To change the choice of printer, either change the default settings in Windows NT or set up your choice of destination printer for the current working session by selecting **File:Printer setup** in the Main menu module and selecting the desired printer.

3.5 Logging off



To log off from UNICORN click on the **Logoff** button or select the **Logoff** menu command.

Processes that are running when you log off will continue to run, and may be left locked with a locking password or unlocked (see Section 6.5 for more details). If the Method editor module was active at the time of logoff, it will be re-opened when the same user logs on again.

UNICORN will still be open after a user has logged off, and another user may log on. We recommend that you always log off when you leave the computer to prevent other users from accidentally changing or deleting your files or disturbing your runs.

3.6 Quitting UNICORN

To quit UNICORN and close the program, select **File:Quit program** in the Main menu. You will be prompted to save any unsaved data in the Method editor or Evaluation module.

Note: You can quit UNICORN during a run which does not use scouting or a MethodQueue without affecting the run. Do not however shut down Windows NT or turn off the computer while the run is in progress.

Introductory material

Methods and runs

Evaluation

System management

Appendices

4 Creating methods from method templates

UNICORN is supplied with a set of method templates which are used as the starting point for creating customised methods. These method templates are defined with variables for critical parameters in the separation, so that customised methods can be created for most purposes simply by setting appropriate values for the method variables. Different templates are provided for different system strategies. This chapter describes how to create and edit methods at this level. See Chapter 5 about advanced method editing facilities.

Note: Method templates are only available for ÄKTAdesign systems and BioPilot delivered with standard strategies.

Briefly, the steps in creating a method by editing method variables are as follows:

1. Click on the **New Method** toolbar button in the Main menu, or select **File:New:Method** in the Main menu or **File:New** in the Method editor module. Select a system, technique, template and column.
2. Choose **View:Run setup** or press the **Run setup** button.
3. Adjust the values for the method variables.
4. Read the method notes.
5. Save the method.

Note: The fastest and easiest way to run a method is to click on the **Instant Run** toolbar button in the Main menu. This function runs a method template and the method is not saved in the Main menu **Methods** window. The method may, however, be recovered from the result file.

4.1 Creating a new method

To create a new method, do one of the following:



- click on the **New Method** toolbar button in the Main menu
- select **File:New:Method** in the Main menu

- click on the **New Method** toolbar button in Method editor



- select **File:New** in the Method editor

These alternatives are equivalent. When you choose the command from the Main menu, the Method editor is opened automatically.

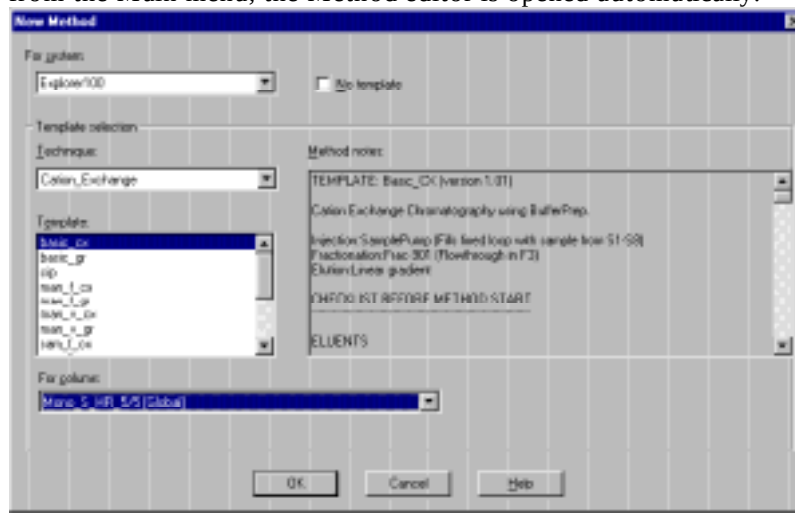


Figure 4-1. The New method dialogue.

1. Choose the system for which the method is intended.

   The instructions available for a given system are determined by the system strategy. A method developed for one system may not be valid on another.

2. Select a chromatographic technique. The templates available for the selected technique will be displayed.

   Available techniques may include **Anion_Exchange** chromatography, **Cation_Exchange** chromatography, **RPC** (reverse phase chromatography), **HIC** (hydrophobic interaction chromatography), **Size_Exclusion** chromatography and **Affinity** chromatography. Selection of **Any** causes the templates for all techniques to be displayed. For BioPilot, **Any** must be selected.

3. A list of ready-to-run method templates is displayed for the selected technique. Available templates are determined by the system strategy. Select one of these templates to create customised methods either by adjusting variable values (see Chapter 5) or changing method instructions.

Click on a template to display information about the particular template in the **Method notes** field.

For some ÄKTAdesign systems, a basic template is available for each technique that is suitable for the first run, in addition to a number of more advanced templates which you can use when you are familiar with using the basic template. Some of the more advanced templates are named according to the following abbreviations (**xxx_y_zz**).

The first three letters (**xxx**) identify the type of sample application used:

man	the sample loop (or Superloop) is filled manually with a syringe
sam	the sample loop is filled automatically with the sample pump
sys	the sample is applied directly through the buffer pump
aut	an autosampler is used to apply the samples

The letter in the middle (**y**) identifies the type of fractionation used:

f	the fraction collector is used with the fixed volume collection
p	the fraction collector is used with peak collection
v	fractions are collected with the outlet valve (useful for larger volumes)

The last two letters (**zz**) only identifies for which technique the template is written.

The VarpH template is available for some systems and can only be used in ion exchange chromatography for pH scouting using BufferPrep. Depending on the system, the CIP (cleaning-in-place) template is used for cleaning the column automatically with up to 5 different solutions.

4. Choose a specific column to be used. Only columns for the selected technique are displayed. If you do not find your specific column it can be added to the list (see Section 5.9). The column volume, recommended flow rate, pressure limit and averaging time for the selected column will be automatically copied into the method thus reducing the need to edit the method. For BioPilot and BioProcess, only the column volume will be copied into the method.

Note 1: You may have to adjust the pressure limit depending on the configuration (tubing etc.) of your system. See the on-line **Adviser** for more details.

Note 2: Except for the column volume, the column-specific values will only be copied into the method if the corresponding instructions are available as variables.

If **Any** is selected, you can use any column but must enter the column volume in the method on the Variables page. It is recommended that a specific column is selected.

5. Click on **OK** once you have made your selections. The method template will now be opened in Run setup view as an untitled method.

4.2 Editing method variables

The method templates are constructed from blocks representing the stages in a typical separation. Each block has a set of method variables, displayed on the Variables page in the run setup. You set default values for the variables in the Method editor, and can change these values for a particular run in the start protocol before the run is started.



Figure 4-2. The Variables page in Run setup.

Work through the variable list, adjusting the values to suit your separation. To change a variable value, simply type the required value in the field. Remember that the values you enter here will be default values, suggested each time the method is run.

If the whole variable list does not fit on one screen, a scroll bar will be shown to the right of the list. Click on the arrows at the ends of the bar to scroll one variable at a time, or on the bar itself to scroll one screen at a time. You can also drag the slider button to scroll, but this is not recommended since you can easily miss variables by scrolling too far.

Typical blocks are illustrated with the list below, taken from ÄKTAexplorer 100 with a basic ion exchange template. The list is organised according to the blocks in the method, with mentioned variable parameters identified in italics. Other method templates have different structures and variables.

- **Main**
  Defines the *column* and the method base that were chosen in the **New Method** dialogue. The column can be changed here.
- **Start condition**
  Sets the *column position, UV averaging time, wavelengths, pressure limit,* the *starting buffer concentration* (usually 0%B), *BufferPrep pH* and the *flow rate*. The pressure limit and flow rate are automatically set to default values for the column selected.
- **Column equilibration**
  The number of column volumes (CV) to equilibrate the column is set here. If zero is entered, no equilibration will take place.
- **Load sample loop**
  Select from which inlet (S1-S7) on the sample valve the sample should be drawn. Place the tubing from that inlet into a test tube with the sample. For complete filling of the sample loop, enter a value of 2-5 x the volume of the sample loop in the **Fill_loop_with** field.
- **Sample injection**
  Empty the sample loop with 5 x the volume of the sample loop to apply all the sample.
- **Wash out unbound sample**
  If you want to wash out unbound sample from the column with the start concentration of the buffer after the sample has been applied, enter the length of the wash here.
- **Fractionation**
  Set the fraction size during elution. The fraction collector will start at the beginning of the gradient. Zero means that fractions will not be collected and the eluate is diverted to F1.

- **Gradient**
  The target concentration of eluent B and the length of the gradient is set here. A linear gradient will be created.
- **Clean after gradient**
  The concentration of eluent B to clean the column with after the separation is set here (usually 100 %). The length of the cleaning is also set.

Note 1: The base for the all the blocks in the method templates except start conditions and sample injection is column volume. To use absolute volume or time as the method base, change the Base instruction in the Main block using the Text instruction editor. See Chapter 5 for a description of how to do this. For blocks where the length of the block is defined as a variable (e.g. sample loading, column wash), setting the length to zero will effectively skip the block. Instructions in the block will still be executed, but in an immediate sequence of zero duration.

You can get a graphical view of the gradient profile and the length of the blocks in a method by viewing the **Gradient** window in the Method editor (**View:Windows** and **Gradient**) or in the **Gradient** page of Run setup.

Click on the x-axis button in the graphical display to change a base for the graphical display. Changing the display base will not affect the base in the method.

4.3 Method notes



Click on the **Notes** thumb-tab in the Run setup to show the **Notes** page, and read through the method notes. You can maximise each section in the notes page to fit more of the text on one screen. Click on the printer icon or choose **File:Print** to print the method notes.

The method notes provided with each template describe the important information about the template and, if relevant, how the system should be connected for the method to work correctly. If your system does not correspond to the description, either rearrange the valves and tubing connections in accordance with the method notes description or edit the method instructions (see Chapter 5) in accordance with your system setup.



Figure 4-3. The Notes page in Run setup with the method notes maximised.

4.4 Saving the method

A new method created from a method template is untitled, and must be saved under a method name before it can be run. Click on the **Save Method** toolbar button or choose **File:Save** to save the method.

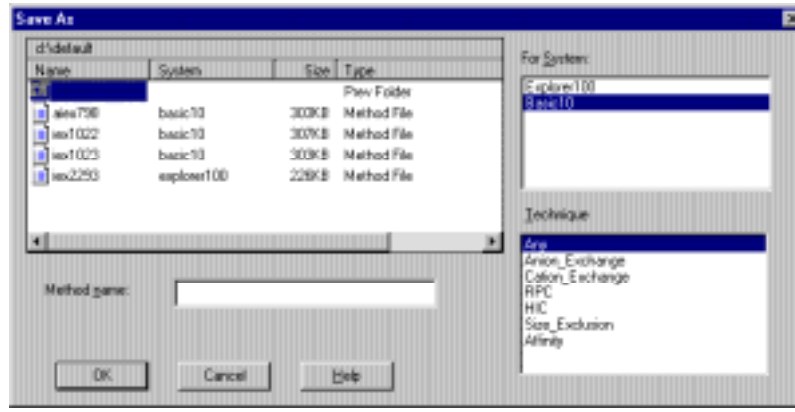


Figure 4-4. Dialogue for saving a method.

1. If required, select another folder than the default home folder in which to save the method.

2. Enter a **Method name** for the method. Method names may be up to 256 characters long. The method name must be unique for the chosen system within the folder (see steps 2 and 3).

3. If you have more than one system connected to the computer, choose the **System** for which the method is intended. The method can only be run on the system for which it is saved. Remember that different systems may have different configurations and control capabilities.

Note: Each method is written for a specific strategy. The function of the method cannot be guaranteed on systems having other strategies.

4. Choose the **Technique** for which the method was written.

5. Click on **OK**.

Note: The method templates are written for standard strategies. If you receive a syntax error message when the method is saved, one or more instructions in the method are invalid. These may be calls to blocks which are not defined, or instructions which are invalid in your customised strategy (this can also arise if a method is written for one system and saved for another). Invalid instructions are marked in red in text instruction mode in the Method editor (see Section 5.4.1), and must be deleted or replaced before the method can be run.

The method remains open in the Method editor when it has been saved, so that you can continue editing if you wish. Once the method has been saved, choosing **File:Save** saves the current state of the method under the same name. If you want to save a copy of the method under a new name, choose **File:Save As** and enter the details as described above.

4.5 Starting a run

This section briefly summarises how to start a run with a method. The method must be named and saved before it can be started. See Chapter 6 for more details of how to run a method.

Note: If you are editing the method in the Method editor and have made changes that you have not yet saved, these changes will not apply during the run. Similarly, if you edit the method while it is running, the run will not be affected. It is the version of the method that is saved on disk at the time when the method is started that controls the run.

1. Establish a control mode connection to the system where the method is to be run. See Section 6.5 for details. You cannot start a method without a control mode connection to the appropriate system.

2. Place the tubing from the sample inlet selected in the method into a test tube of sample or fill the sample loop or Superloop manually.

3. Choose **File:Run** from System control for the required connection and select the method to run. Alternatively, click on the method in the **Methods** window of the Main menu and select **Run** from the right mouse button menu. Do *not* double-click on the method icon in the Main menu as this will open the Method editor.

4. Change the method variable values if required. The suggested values are those saved in the method. Any changes you make will apply only for the current run, and will be recorded in the run documentation.

5. Go through the rest of the Start protocol, entering information where appropriate. Use the **Next** and **Back** buttons to move through the Start protocol. If you click on **Cancel** on any page in the start protocol, the method will remain loaded in the System control module but will not start. Start protocol pages for most method templates are:

   Variables Adjust variable values as required for the run.

   Questions Fill in answers to the questions.

   Notes Read the method notes and enter start notes if required.

   Evaluation Procedures Select the **print_chromatogram** procedure if you want the results to be printed automatically.

   Result Set the result file name and path (folder) as required. The default result file name includes a 2-digit serial number.

6. The last page of the start protocol has a **Start** button. Click on this button to start the run.

4.6 Editing text instructions



Methods for most purposes can be created by adjusting the method variable values as described above. The method is actually programmed as a series of instructions that use these variables as parameters. To see and/or change the instructions, click on the **Text Mode** button on the Method editor toolbar or select **View:Text instructions** from the menu. To see an overview of the block structure and the gradient in the method, select **View:Windows:Block** and **View:Windows:Gradient** respectively in the Text instruction window (see Section 5.4).

With the Text instruction editor, you have complete facilities for designing and editing your own customised methods. You will also use the Text instruction editor for refining and modifying methods based on the standard templates, e.g.

- Changing the method base (column volume, volume or time).
- Changing valve specifications for inlet and outlet (if the templates do not suit your system configuration).
- Adding or removing variables.
- Adding conditional events (such as starting fraction collection when a peak is detected).
- Adding or removing instructions to change the method functionality.
- Adding or removing blocks to change the method structure.

To gain an understanding of how method templates are built up and can be modified, work through Chapter 5 which gives a full description of method editing facilities.

5 Creating and editing methods

This chapter describes the complete facilities for creating and editing methods in UNICORN. Refer to Section 2.3 for an overview of method concepts. For many applications, suitable methods can be created by changing the default variable values in one of the templates supplied with UNICORN (see Chapter 4). Use the more advanced editing facilities described here for:

- changing selected instructions in the method templates, e.g. changing the outlet valve position
- adding blocks and instructions, e.g. Watch instructions
- changing method instructions to adapt to non-standard system configurations
- creating new methods for applications which are not covered by the templates supplied

Briefly, the steps in creating a method using advanced method editing facilities are as follows:

1. Click on the **New Method** toolbar button in the Main menu, or select **File:New:Method** in the Main menu or **File:New** in the Method editor module.



2. Select a system, technique, template and column. Choose **View:Text instructions** or press the **Text mode** button.

3. Create any blocks that you need (Section 5.3).

4. Enter instructions in the new blocks, and/or modify instructions in existing blocks (Section 5.4).

5. Define variables as required (Section 5.5).

6. Save the method.

5.1 Creating a new method

To create a new method, do one of the following:

- click on the **New Method** toolbar button in the Main menu
- select **File:New:Method** in the Main menu
- click on the **New Method** toolbar button in Method editor
- select **File:New** in the Method editor

These alternatives are equivalent. When you choose the command from the Main menu, the Method editor is opened automatically.

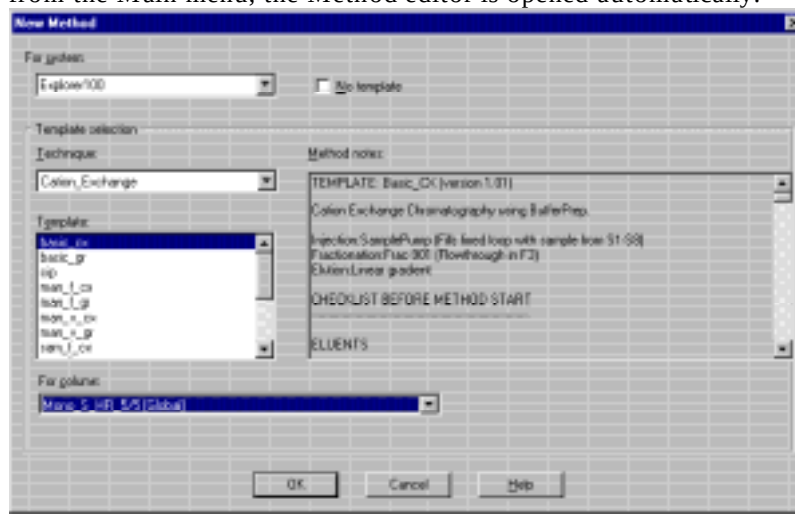


Figure 5-1. The New method dialogue.

1. Choose the system for which the method is intended.

   The instructions available for a given system are determined by the system strategy. A method developed for one system may not be valid on another.

   If you select (check) the **No Template** box, no technique, template or column selections can be made. You will instead receive an empty template which contains only a Main block with a Base instruction. Use this template for developing methods "from scratch".

2. Select a chromatographic technique. The templates available for the selected technique will be displayed.

Available techniques may include **Anion_Exchange** chromatography, **Cation_Exchange** chromatography, **RPC** (reverse phase chromatography), **HIC** (hydrophobic interaction chromatography), **Size_Exclusion** chromatography and **Affinity** chromatography. Selection of **Any** causes the templates for all techniques to be displayed. For BioPilot, **Any** must be selected.

3. A list of ready-to-run method templates is displayed for the selected technique. Available templates are determined by the system strategy. Select one of these templates to create customised methods either by adjusting variable values (see Chapter 4) or changing method instructions.

   Click on a template to display information about the particular template in the **Method notes** field.

   For most ÄKTAdesign systems, a basic template is available for each technique that is suitable for the first run, in addition to a number of more advanced templates which you can use when you are familiar with using the basic template. Some of the more advanced templates are named according to the following abbreviations (**xxx_y_zz**).

   The first three letters (**xxx**) identify the type of sample application used:

man	the sample loop (or Superloop) is filled manually with a syringe
sam	the sample loop is filled automatically with the sample pump
sys	the sample is applied directly through the buffer pump
aut	an autosampler is used to apply the samples

   The letter in the middle (**y**) identifies the type of fractionation used:

f	the fraction collector is used with the fixed volume collection
p	the fraction collector is used with peak collection
v	fractions are collected with the outlet valve (useful for larger volumes)

   The last two letters (**zz**) only identifies for which technique the template is written.

The VarpH template is available for some systems and can only be used in ion exchange chromatography for pH scouting using BufferPrep. Depending on the system, the CIP (cleaning-in-place) template is used for cleaning the column automatically with up to 5 different solutions.

4. Choose a specific column to be used. Only columns for the selected technique are displayed. If you do not find your specific column it can be added to the list (see Section 5.9). The column volume, recommended flow rate, pressure limit and averaging time for the selected column will be automatically copied into the method thus reducing the need to edit the method. For BioPilot and BioProcess, only the column volume will be copied into the method.

Note 1: You may have to adjust the pressure limit depending on the configuration (tubing etc.) of your system. See **Adviser** for more details.

Note 2: Except for the column volume, the column-specific values will only be copied into the method if the corresponding instructions are available as variables.

If **Any** is selected, you can use any column but must enter the column volume in the method on the Variables page. It is recommended that a specific column is selected.

5. Click on **OK** once you have made your selections. The method template will now be opened in Run setup view as an untitled method.

5.2 Method editor interface

5.2.1 Method editor modes

The Method editor interface operates in two modes:

- Text instruction editor
- Run setup editor

Switch modes using the toolbar buttons or the commands in the **View** menu.



Figure 5-2. Method editor toolbar

Icon	*Menu command*	*Function*
	**View:Text instructions**	Open the text instruction editor
	**View:Run setup**	Open the Run setup editor

5.2.2 Text instruction editor



The text instruction editor is used for entering and editing method instructions. Click on the **Text instructions** toolbar button or choose **View:Text Instructions** to display the text instruction editor.

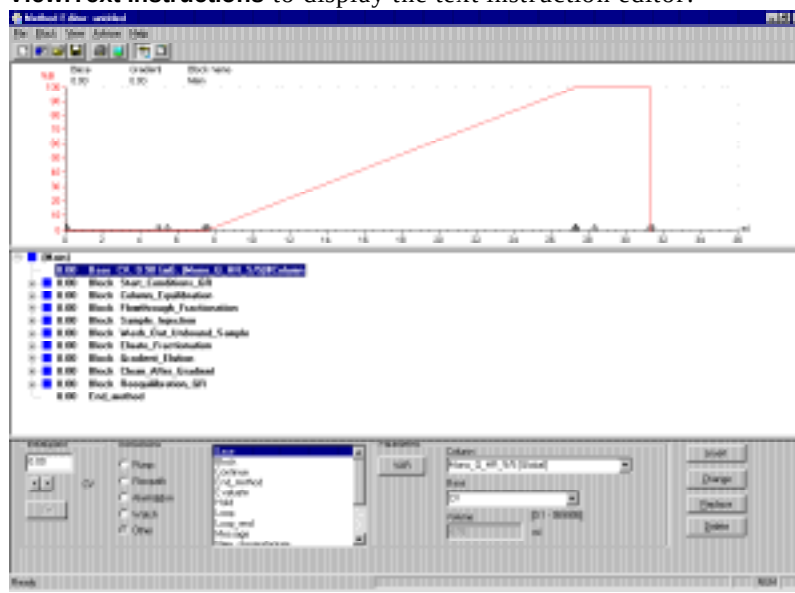


Figure 5-3. The Method editor in text instruction mode, showing the Gradient window (top), text instruction window (centre) and instruction box (bottom).



Up to four windows can be displayed together with the instruction box. Click on the **View Windows** toolbar button or choose the **View:Windows** menu command to open a dialogue for choosing which windows to display.

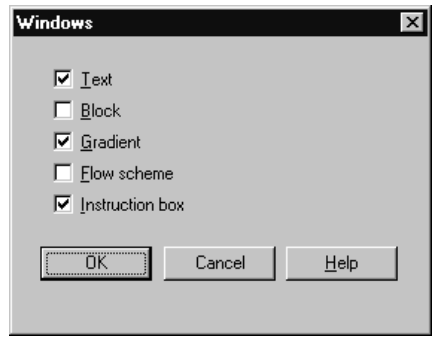


Figure 5-4. The Windows dialogue for selecting which windows to display in the Method editor.

Method editing operations which can be performed in the various windows are summarised in the table below.

Window	*Operations*	*See Section*
Block window	Select block.	5.3
Text window	Display and hide block instructions. Select current instruction. Move instructions within a breakpoint.	5.4
Gradient window	Display block duration and eluent gradient throughout the method.	5.4.5
Flow scheme window	For information only. The flow scheme picture is static and is therefore not updated according to system status or changes in the method.	5.4.6
Instruction box	Specify breakpoints, instructions, parameters and variables. Insert, change and delete instructions.	5.4

5.2.3 Run setup



The Run setup is a series of pages for defining the method properties. To access the Run setup pages, press the **Run setup** toolbar button or select **View:Run Setup** from the menu.



Figure 5-5. Run setup with the Variables page displayed.

To access a given page in the Run setup, click on the relevant tab. Pages in the Run setup editor are described in Section 5.6.

5.3 Method blocks

Method blocks represent "modules" in the structure of the method. Most commonly, each block handles one functional unit within the method (e.g. column equilibration, sample loading, elution etc.). Blocks are "called" either from Main (the top level of the method) or from other blocks using the Block instructions (unconditional) or Watch instructions (conditional) (see Section 5.8.8). Calling a block executes the instructions in the block.

Use text instruction mode to view and edit blocks.

5.3.1 Viewing blocks

In the block window

The organisation of blocks in the method is shown graphically in the block window in text instruction mode.

Each block is represented by a grey bar with the block name and the length of the block. The line is shifted down to indicate calls to other blocks. In the example above, the blocks are called in sequence from the Main block at breakpoint 0. Blocks to which there is no valid call are not shown in this window.



Figure 5-6. The Method editor block window.

Conditional (Watch) instructions are indicated by a green line showing the start and duration of the watch. The example above has a Watch instruction to start the fraction collector which is active throughout the gradient elution block. Loop instructions (to repeat a group of instructions) are also indicated in the block window.

In the gradient window

Blocks are represented in the gradient window by marks on the x-axis showing the length of each block. The name of the block in which the cursor line is currently placed is shown at the top of the window (see Section 5.4.5).

In the text instruction window

In the text instruction window, the method is shown as a list of blocks, denoted by the blue squares. Beside each block is also a '+' symbol, which you can click on to expand the method tree to view the instructions for a specific block. To hide the instruction again, click instead on the '-' symbol for that block. Alternatively you can double click on the block name to view or hide the instructions (see Section 5.4.1).

If you click on the line representing a block in the block window, the first instruction in the block will be highlighted in the text window.

5.3.2 Calling blocks

Blocks are called in a method with the Block instruction. When a block is called the instructions in the block are executed in the order that they are written until the block is finished or the End_Block instruction is executed. Any settings made in a block are valid throughout the method until the settings are changed.

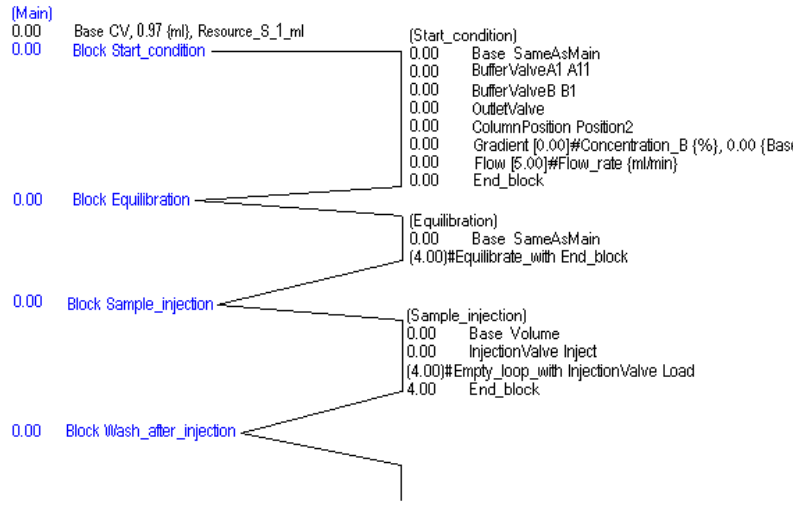


Figure 5-7. Illustration of the flow of process control through method blocks.

Calls may be of two types:

- *Unconditional calls* are made with the Block instruction.
- *Conditional calls* are made with a Watch instruction, which makes it possible to call a specified block or an instruction when a particular monitor signal meets a given condition. As long as the condition is not met, the block is not activated. There are different Watch instructions for each process monitor signal, and each Watch instruction can use various conditions to respond to absolute signal values or to rate of signal change.

  Note that the breakpoint when the Watch instruction is issued determines when the watch begins, not when the block is activated. The block will in fact never be activated if the watch condition is not met during the run.

  Once set, a watch remains active until the condition is met or a new Watch instruction is issued for the same monitor. The watch is cancelled automatically when the condition is met. A watch can also be turned off with the Watch_off instruction.

  See Section 5.8.8 for more details of Watch instructions.

5.3.3 Adding blocks



To add a new block use the Text instruction editor and click on the **New block** toolbar button or select **Block:New**.



Figure 5-8. The New block dialogue.

Block name

Enter a name for the block in the **Name** field. Block names may be up to 30 characters long, and may contain letters (A-Z), digits (0-9) and underscore characters.

Block names must be unique within the method. The case of letters is retained but is not significant (the names Start_Frac and START_FRAC are treated as identical).

Base

Choose a base for the block. If you choose **SameAsMain**, the new block will inherit the base from the Main block in the method. The corresponding Base instruction will be inserted in the block at breakpoint 0. If you choose **CV**, enter a value for the column volume. If you chose a specific column, a column volume is entered automatically.

Length

You can enter a length for the block if required. An End_Block instruction will automatically be inserted in the block at the corresponding breakpoint. This field may not be left blank.

Call

You can call the new block from an existing block (e.g. the Main block). The block is called by an instruction named Block. Choose the block from which the newly created block should be called in the **From** field and enter the breakpoint at which the call is to be made in the **At** field. If you do not want to call the block (e.g. when the block being created is to be activated by a Watch instruction), choose an empty line in the **From** field. These blocks are placed last in the method in the Unused category.

Note 1: If the Block instruction is placed at the same breakpoint as the End_Block instruction, the Block instruction will be placed immediately before End_Block.

Note 2: Do not call a block from within itself. You will generate a potentially infinite loop, which exceeds the maximum number of calls allowed in a method. A loop symbol is displayed at the beginning of the line if this occurs.

Press **OK** to add the new block.

Strategy for creating blocks

For blocks which are to be called unconditionally, you have the option of creating the Block instruction at the same time as you create the block, by selecting where the block will be inserted in the **From** field of the **New block** dialogue.

For blocks which are to be called conditionally with a Watch instruction, first create the block and save it under unused by selecting an empty line. Insert the instruction into the text method and then make a call to the block. The block will move from unused to beneath the watch instruction.

Note: If you call to the block before inserting the watch instruction, a copy of the block will be created, i.e. there will be two instances of the block in the text method.

5.3.4 Deleting blocks

To delete blocks from a method, choose **Block:Delete Block**. The **Delete Block** dialogue is displayed.

Select one or more blocks to delete and press **OK**, then confirm the deletion. Double-clicking on a block name is equivalent to selecting the block and pressing **OK**.

If you delete a block by mistake, you can restore the block by importing from the saved version of the method (see Section 5.3.6) provided that you have not saved the method with the block deleted.

Note: Any Block or Watch instruction that calls a deleted block remains in the method, but is shown with a red bullet beside the instruction line to indicate that it is no longer valid. A method cannot be run if it contains invalid instructions.



Figure 5-9. The Delete blocks dialogue.

To totally delete a block you should in turn select each call to the block in the method and click on the **Delete** button in the text instruction box. This places the block in to the unused group. Next delete the block by selecting **Block:Delete Block** and then selecting the block for deletion in the displayed dialogue.

5.3.5 Renaming blocks

To rename blocks, choose **Block:Rename Block**.



Figure 5-10. The Rename Blocks dialogue

Select the block to rename, enter the new name in the **New Name** field and press **Rename**. The dialogue remains open until you press **Close**, so that you can rename more than one block without closing the dialogue.

If the block you renamed is used in a Block or Watch instruction, the block name in these instructions will be changed automatically.

5.3.6 Importing and copying blocks

You can import blocks from other methods to the method you are developing. You can also use the import function to copy blocks within the method, in which case the imported block should be given a new name.

1. Select **Block:Import Block As**.

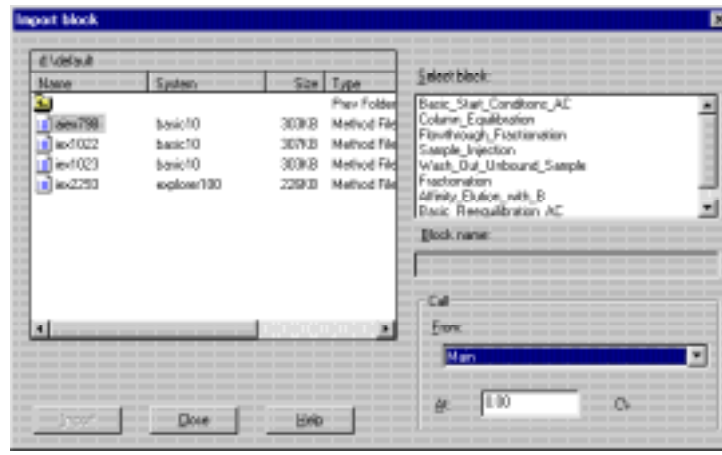


Figure 5-11. The Import Block dialogue.

2. Choose the method from which you wish to import and mark the block to import. The name of the selected block is suggested in the **Block name** field.

3. Select a block from the **Call** drop-down list into which the imported block will be placed and enter a breakpoint value.

4. Click on **Import** to import the block. The dialogue remains open until you click on **Close**, so that you can import more than one block without closing the dialogue.

Note: If you use the import function to copy blocks within a method, the blocks are copied from the saved version of the method on disk. Any changes you have made in the method but not yet saved will not be copied.

The imported block may not have the same name as an existing block in the method. If the default name is not allowed for this reason, the **Import** button will be grey and locked. Change the name of the imported block so that the Import button becomes available.

The block is imported exactly as it appears in the source method. If the base of the imported block is defined as SameAsMain, the block will inherit the main base in the new method, regardless of the base in the source method.

5.4 Method instructions

Use the instruction box in text instruction mode to enter, edit and delete instructions.

5.4.1 Viewing instructions

Instructions are displayed in the text instruction box.

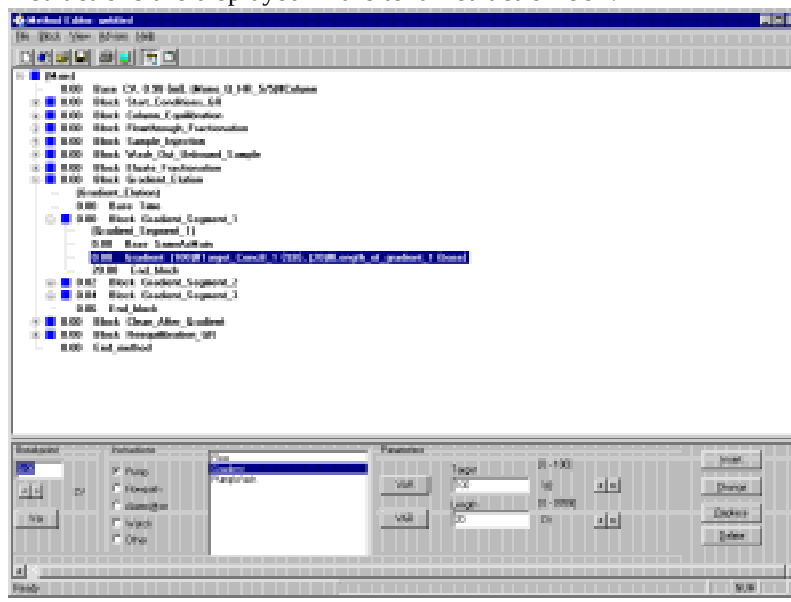


Figure 5-12. The text instruction window (top) with the instruction box (bottom).

Instructions are displayed in the text instruction window as follows:

Blue square beside text	Valid call instructions (i.e. Block and Watch instructions to other blocks in the method).
Bold text	Valid instructions.
Red bullet beside text	Instructions with invalid syntax. These may be: (a) calls to blocks which are not defined in the method, or (b) instructions which apply to a different system strategy (these can arise if a method is written for one system and saved for another). All such instructions must be deleted or changed before a method can be run (see Sections 5.4.3 and 5.4.4).
Normal text	Instructions which will not be executed because they are either after the end of a block or method or constitute a block to which there is no call.
Text with a loop symbol	When a block is called from within itself this will generate a potentially infinite loop, which might exceed the maximum number of calls allowed in a method.

Double-click on a Block or Watch instruction to display or hide the instructions in the called block or click on the '+'/'-' symbol for the block respectively. Double-clicking on the **Main** keyword at the beginning of the method will show or hide instructions in all blocks in the method.

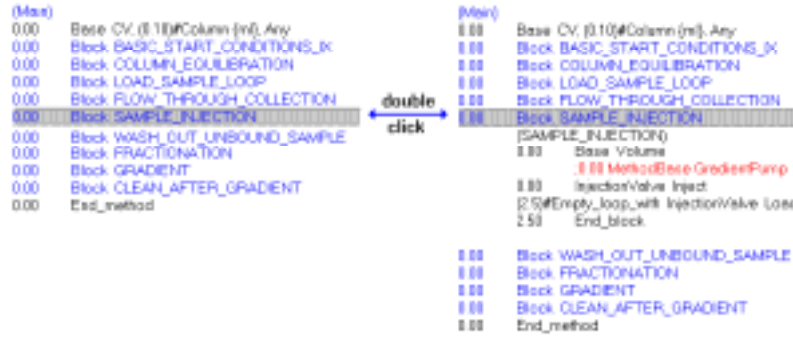


Figure 5-13. Displaying and hiding block instructions.

Instructions are indented for each call level (see illustration above).

5.4.2 Adding instructions

To add a new instruction:

1. In the text instruction window, select an appropriate block and display the instructions within the block.

2. Select an instruction line in the block.

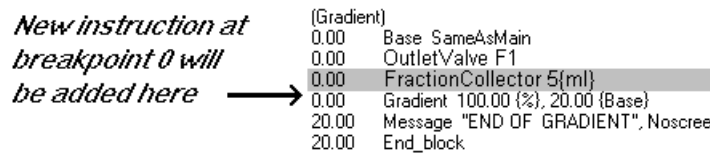

New instruction at breakpoint 0 will be added here
(Gradient)
0.00 Base SameAsMain
0.00 OutletValve F1
0.00 FractionCollector 5{ml}
0.00 Gradient 100.00 {%}, 20.00 {Base}
20.00 Message "END OF GRADIENT", Noscreen
20.00 End_block

Figure 5-14. Instructions added at the same breakpoint as existing instructions are inserted after the highlight.

Note: Make sure that the selected instruction line is within the block, not the call to the block.

3. New instructions are added from the Instruction box. Open the Instruction box if not already displayed (**View:Windows**). For the new instruction:

   (a) Set the desired breakpoint in the **Breakpoint** field.

   (b) Choose the instruction type from the five options and select the desired instruction from the displayed list. For short help on the purpose of each instruction, click on the instruction and press <F1>.

   (c) Enter values for instruction parameters in the **Parameters** field. If a scroll bar appears on the right-hand side of the **Parameters** field, additional parameters are required.

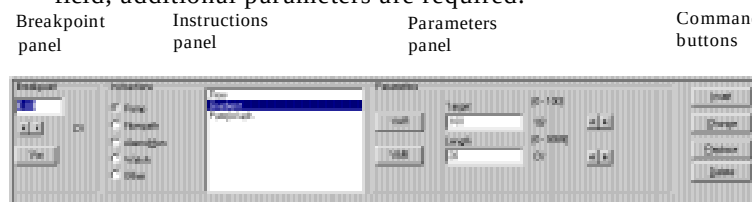


Figure 5-15. The Method editor Instruction box.

4. Click on **Insert**. The new instruction will be inserted in the block either

   (a) at the position of the breakpoint of the new instruction if there are no other instructions at that breakpoint

   (b) immediately after the currently highlighted instruction if the highlight is at the same breakpoint as the new instruction

   (c) as the last instruction at the breakpoint if there are several instructions at the same breakpoint as the new instruction and none of these is highlighted.

Note 1: Instructions that are placed at the same breakpoint are executed simultaneously, with the exception of Block instructions which are executed in the sequence in which they are written.

Note 2: If you are using ÄKTA systems, the Pause instruction will stop execution at this breakpoint, i.e. instructions following after Pause at the same breakpoint will not be executed.

5.4.3 Deleting instructions

To remove an instruction:

1. Select the instruction in the text instruction window.

2. Press **Delete** in the Instruction Box or press on the <Delete> key.

An instruction that has been deleted can only be recovered by re-inserting the instruction. If you want to suspend execution of an instruction temporarily (e.g. during development work), you can replace the breakpoint with a value after the End_block or End_method instruction. Any instructions after the end of a block or method will not be executed.

Note: You cannot delete the Base instruction at the beginning of a block.

CAUTION
If you delete the End_block instruction, the block will end at the last instruction in the block. If a gradient is currently being formed, the gradient will continue into the next block (see Section 5.4.4).

5.4.4 Changing instructions

There are three possibilities for changing an instruction:

- change the breakpoint
- change parameters (including variables, see Section 5.5)
- select another instruction

To change an instruction:

1. Select the instruction in the text instruction window. The instruction with its current parameters will appear in the Instruction Box.

2. In the Instruction Box, make the required changes to the breakpoint or parameters or choose a new instruction.

3. Press **Change** or **Replace**. These buttons are equivalent unless changes are made to the breakpoint or the length of a gradient

Changing breakpoints

Change and **Replace** have different functions if the breakpoint is changed:

- **Change** shifts all subsequent instructions in the block according to the change in the breakpoint. **Change** does not affect the relative order of instructions in the method. You cannot change the breakpoint of an instruction to earlier than the nearest previous breakpoint in block. For example, changing FractionCollector from breakpoint 0 to 5 with **Change**:



Figure 5-16. Change moves the selected instruction and all subsequent instructions.

- **Replace** moves the selected instruction but does not change the breakpoint of any other instruction. **Replace** can change the relative order of instructions in the method. For example, changing FractionCollector from breakpoint 0 to 5 with **Replace**:

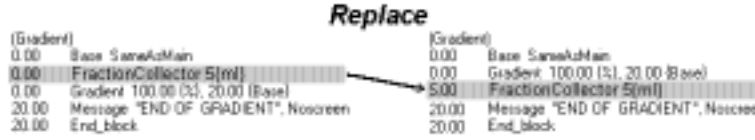


Figure 5-17. Replace moves only the selected instruction.

Changing gradient lengths

The length of a gradient is changed by altering the **Length** parameter in the Gradient instruction. If **Change** is used to alter the length of a gradient, the breakpoints for any instructions issued during the course of the gradient will be adjusted proportionately, so that they are always placed at the same relative position within the gradient. Instructions issued after the end of the gradient will be shifted by the amount of the change. Since the gradient functions over time, any instruction that you want to insert after a gradient should be placed after the combined breakpoint and gradient length.

Note: Moving the End_block instruction in a gradient block with **Change** does not affect the length of the gradient.

If **Replace** is used to alter the length of a gradient, other instructions are not affected.

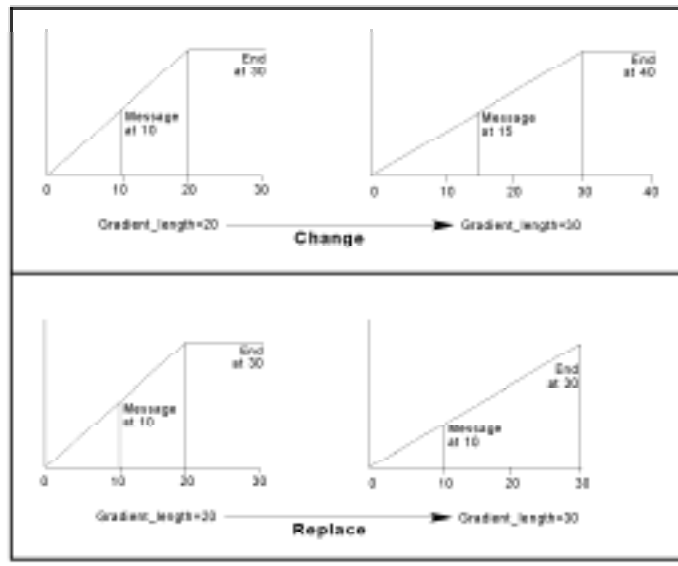


Figure 5-18. Illustration of the different effects of Change and Replace on instructions within and after gradients.

Moving instructions within a breakpoint

To change the order of instructions within the same breakpoint in a block, mark the instruction to move with the left mouse button and drag the instruction to its new location, holding the left mouse button down. You can only move instructions in this way within a group of instructions at the same breakpoint.

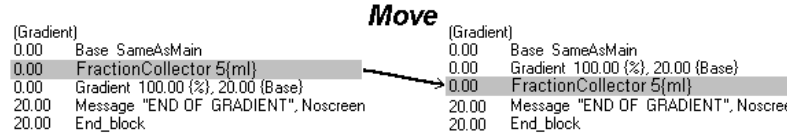


Figure 5-19. Instructions can be moved within the same breakpoint by dragging with the right mouse button.

5.4.5 Using the gradient window

The gradient window provides a graphical overview of the block structure and eluent gradient in the current method:

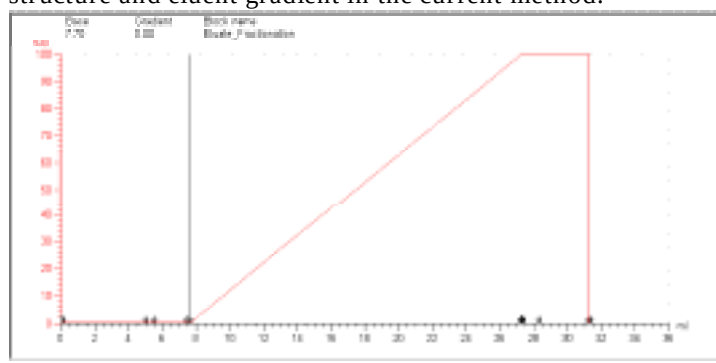


Figure 5-20. The Method editor gradient window.

Vertical cursor line

A vertical cursor line can be dragged from the y-axis with the mouse. As you drag the cursor line:

- The current position is identified at the top of the window, in terms of the block name, x-position in the currently displayed base and eluent concentration in % eluent B. The duration of variable length blocks are based on the default variable values.
- If the text instruction window is displayed, the instructions are scrolled so that the instruction at the cursor line is at the top of the window.
- If the Instruction box is displayed, the instruction at the cursor line is shown in the dialogue.

The zoom function

To zoom in on a selected region of the Gradient window:

1. Press down and hold the left mouse button and drag a rectangle out on the screen to encompass the area to be viewed.

2. Release the mouse button. The display is now zoomed in on the selected area.

3. Repeat the process for further magnification of selected areas.

You can reduce the scale of the zoom in function in two ways, either:

- reverse each zoom in action a step at a time by displaying the right mouse button menu and selecting **Undo Zoom**, or,
- reverse all of the zoom in actions to the default scale setting by displaying the right mouse button menu and selecting **Reset Zoom**

Changing the base

You can select the base shown on the x-axis, either time, volume or column volumes. Make the appropriate selection from the right mouse button menu option **Base type**. Changing the base for the display does not affect the base in the method instructions. You can therefore check how long a method will take simply by setting the axis scale to time, even if the method blocks are written in volume or column volume base.

Viewing hatch marks

You can display a grid in the Gradient window by selecting **Hatch** from the right mouse button menu.

5.4.6 The flow scheme window

The flow scheme window displays the configuration of system components. This window is static and for information only, useful for example in identifying valves for flow path instructions.

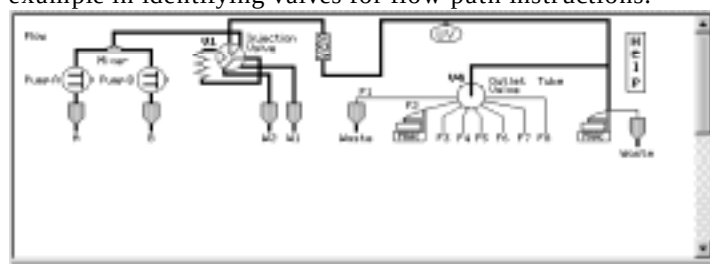


Figure 5-21. The Method editor flow scheme window.

5.5 Method variables

Variables can be assigned to many instruction parameters even including breakpoints, although cannot be assigned, for example, to watch conditions and BufferPrep recipe. Variable values can be changed immediately before the start of a run without using the Method editor, allowing one method to be used for runs under a variety of conditions (see Chapter 4). Variables also form the foundation for automatic method scouting (see Chapter 7).

Variables are defined with names which can be explicit descriptions of the variable function, e.g. Sample_volume, Gradient_length. Suitable choice of variable names can make the method easier to read and understand, and also help the operator in setting variable values at the start of a run.

Each parameter defined as a variable is also assigned a default value, which is used if no changes are made to variable values at the start of a run. Up to 64 variables can be defined in a single method.

5.5.1 Identifying variables

Parameters defined as variables are identified in two ways:

- in the text instruction window, the parameter is given as the default value in parentheses followed by the variable name, e.g. (4.00)#`Empty_loop_with`.

```
(Sample_injection)
0.00    Base Volume
0.00    InjectionValve Inject
0.00    OutletValve F2
{4.00}#Empty_loop_with InjectionValve Load
4.00    End_block
```

Figure 5-22. Default values for variables appear in parentheses in Text instructions

- when the instruction is shown in the Instructions box, the **Var** button beside the parameter field is enabled.

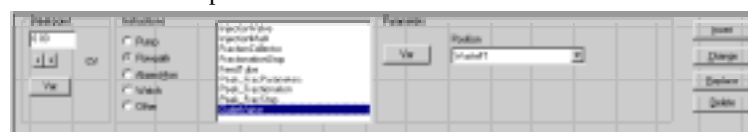


Figure 5-23. Parameters with variable definition are identified by an enabled **Var** button.

All variables are also listed on the Variables page of the Run setup (see Section 5.6.1), grouped according to the block in which they appear.

5.5.2 Defining variables

To define a new variable (i.e. convert an existing fixed value to a variable):

1. In the Text instructions window, select the instruction where you want to define the variable. The parameters for the instruction are shown in the instruction box.

2. Locate the breakpoint or the required parameter in the instruction box. Click on the Var button.

3. Enter a name for the variable in the dialogue and click on **OK**.

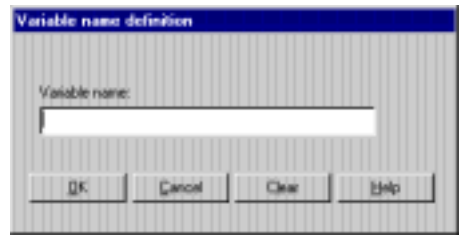


Figure 5-24. The Variable name definition dialogue.

Variable names may be up to 20 characters long, and may contain letters (A-Z), digits (0-9) and the underscore character. Use underscore characters instead of spaces if you want to separate words in a name (e.g. Flow_rate). Names must be unique within the method.

The case of letters is retained but is not significant. The names Flow_Rate and FLOW_RATE are treated as identical.

When you define a variable, the value in the parameter field applies as the default value for the variable.

Note: Only one variable which affects block length (breakpoint or gradient length) may be defined within each block. Any number of other parameters may however be defined as variables within a block.

Default variable values can be changed either by editing the instruction in the Instruction box or by changing the value in the Variables page of Run setup. Changes made in the text instruction are automatically updated in the Variables page and vice versa (Figure 5.26).

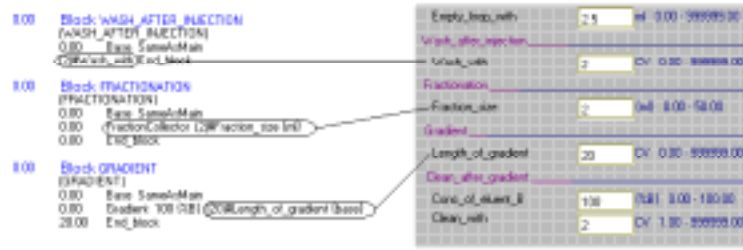


Figure 5-25. Relationship between variables in text instructions and in the Variables page of Run setup.

Note: If a breakpoint or gradient length is defined as a variable, changing the variable value when the method is started will shift other instruction breakpoints accordingly. This functionality is equivalent to using **Change** to alter a breakpoint or gradient length (see Figure 5-16 for how **Change** affects instructions within gradients).

5.5.3 Removing a variable

To convert a variable to a fixed value:

1. In the text instruction window, select the instruction where you want to remove the variable. The parameters for the instruction will be shown in the instruction box.

2. Locate the required parameter in the Instruction box. Press the **VAR** button.

3. Click on **Clear** to delete the variable name and click on **OK**.

Note: Do not remove column variables (flow, pressure, UV averaging time) if you want these parameters to be updated if you change column.

5.5.4 Renaming a variable

To change the name of an existing variable:

1. In the Text instruction window, select the instruction where you want to rename the variable. The parameters for the instruction will be shown in the instruction box.

2. Locate the required parameter in the instruction box. Press the **VAR** button.

3. Enter a new variable name in the dialogue and click on **OK**.

5.6 Run setup



The Run setup is a series of pages for defining the method properties. To access the Run setup pages, press the **Run setup** icon at the top of the Method editor toolbar. To access a given page in the Run setup, click on the respective tab.

5.6.1 Variables

The Variables page lists all variables used in the method with their default values, organised by method block. You can change the default values to create a variant of the method (see Chapter 4). Note that in a scouting method, values for variables included on the scouting page cannot be changed on the Variables page.

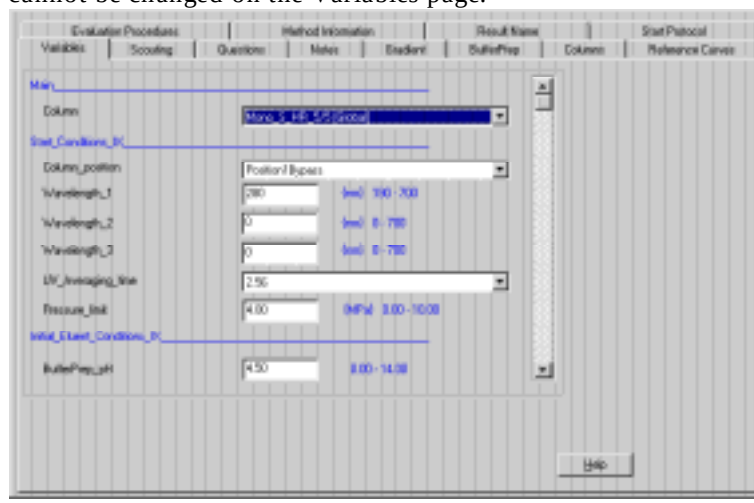


Figure 5-26. The Variables page in Run setup.

To change the default values, simply enter new values in the appropriate fields. Use the scroll bar to display additional variables if the variables occupy more than one screen. Click in the scroll bar to move one screen at a time, or on the arrows to move one variable at a time.

The changed values will be displayed for the corresponding instructions in the text instruction window. Remember to save the method with the changed variables.

Note: The **Variables** box must be checked in the Start protocol if you want to be able to change variable values at the start of a method.

5.6.2 Scouting

A scouting scheme is a series of potential runs where chosen variable values are varied. When a method is run using scouting, the method is repeated automatically for each selected run in the scouting scheme. Typically, scouting will vary one or more variables in a series of runs, e.g. flow rate or elution gradient. Method templates for scouting methods are supplied with UNICORN. See Chapter 7 for a full description of scouting facilities.

All scouting parameters can be set when a run is started, provided that **Scouting** is checked in the Start protocol. The parameter values entered in the Method editor provide the default settings for the scouting method.

To prepare a method for scouting:

1. Select a template method or create a custom method with appropriate variables in the method instructions. Templates are included for common scouting purposes, but any template can be used.

2. Open the Scouting page in Run setup.

3. The **Scouting Variables** dialogue is displayed automatically if there are no scouting variables defined. If scouting variables are already defined and you want to change the selection of scouting variables, click on the **Define** button to display the **Scouting Variables** dialogue.

   Select the required variables in the variable list.

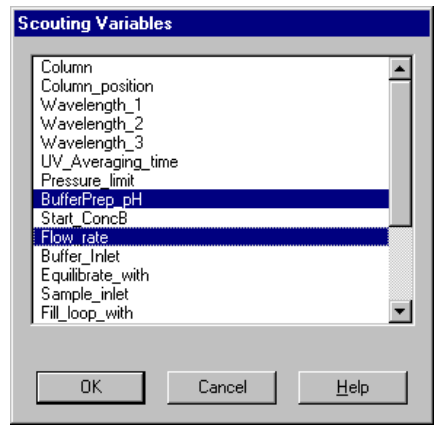


Figure 5-27. The dialogue for defining scouting variables.

3. Click on any cell in the first run column in the scheme. This inserts the default values for all variables selected under **Define**. Make any changes you require in the variable values. For variables with text values (e.g. column position), double-click on the variable field and select the required value from the list that appears.

Note: Values for variables selected for scouting under **Define** cannot be changed on the Variables page. Changes made on the Scouting page do not affect the default values in the method instructions.

4. Click on the next run to copy the values from the first run column, and change variable values as required.

5. Repeat this procedure until you have defined all the runs you require. The example below shows scouting set for four flow rates.

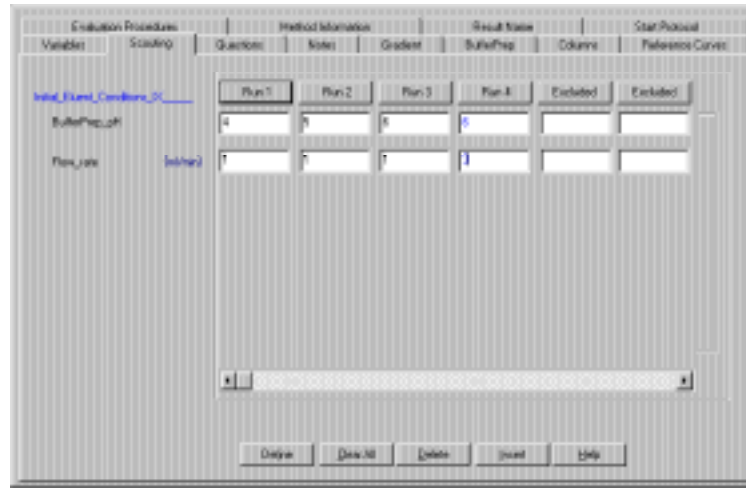


Figure 5-28. Scouting scheme with varying BufferPrep_pH and flow rate in this example.

You can define up to 99 runs in a scouting scheme. Use the scroll bar at the bottom of the page to define more than 6 runs. Each run can be set to **Run** or **Excluded** by clicking on the button at the top of the column of variable values in the scouting scheme definition. Values can also be changed during the run in System Control Run setup

All variables in a used set must be assigned values.

Any changes that you make to variable values when a scouting scheme is run are saved in the result file. Results from a scouting run are saved in a scouting folder (see Chapter 7).

Inserting scouting runs

To insert a new scouting run before an existing run, click on any variable on the scouting page and then click on **Insert**. The new run will inherit the variable values from the preceding run or from the default values in the method if the run is inserted at the beginning of the scouting series.

Deleting scouting columns

To remove one run from the Scouting page, click on any variable in the run and then click on **Delete**. Confirm that you want to delete the run.

To clear all runs, click on **Clear All** and confirm that you want to clear them all.

Start protocol for scouting runs

On the Start protocol page (see Section 5.6.10), you may choose whether to display the Start protocol at the beginning of the first run only in a scouting series or at the beginning of every run. Displaying the Start protocol at the beginning of the first run only allows unattended operation of the entire scouting series, but does not permit variable values and other settings to be adjusted at the beginning of each run. Displaying the start protocol at the beginning of every run requires that you press the **Start** button to start each run.

5.6.3 Questions

Questions provide a means for entering structured run-specific information at the start of a run. Method templates supplied with UNICORN are defined with a set of questions for sample, column and eluent identification. To define questions which will be shown when the method is started, open the Questions page in Run setup.

Note: For questions to be shown in the start protocol, the **Questions** option must be checked in the Run set up Start Protocol page.

Questions may have the following status:

- **Mandatory**: these questions must be answered before a method is started.
- **Authorised**: answers to these questions must be acknowledged by a user with **Confirm/Unlock** authorisation (see Section 14.2). The user's password must be given to acknowledge the answers.

- **Chromatogram**: these questions will be printed with the answers on the same page as the chromatogram if Diagram header is chosen in an evaluation report (see Section 9.5).

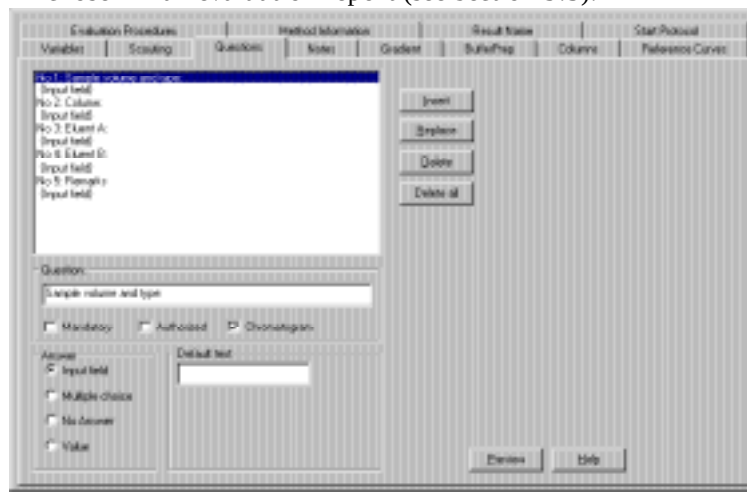


Figure 5-29. The Questions page in Run setup

Questions may be defined to accept four types of answers:

- **Input field** accepts any alphanumerical input as the answer. Input field questions may have a default answer. Example

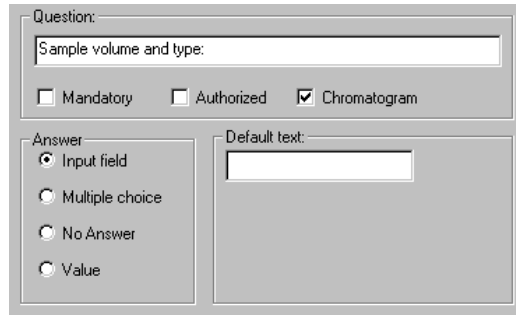


Figure 5-30. Options for input field questions.

- **Multiple choice** allows the user to choose one of a defined set of answers. To allow a blank answer, enter a space in one of the pre-defined answers. Example:

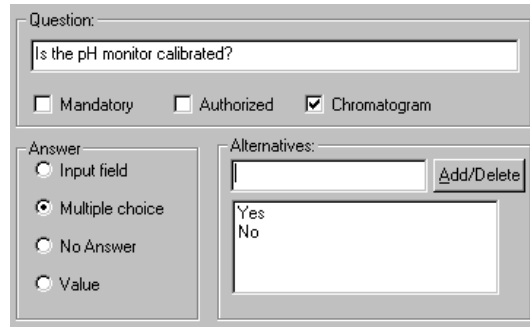


Figure 5-31. Options for multiple choice questions.

- **No answer** does not require an answer. This kind of "question" may be to display important information or to split a question over more than one line (by setting all but the last line to **No answer**). Example:

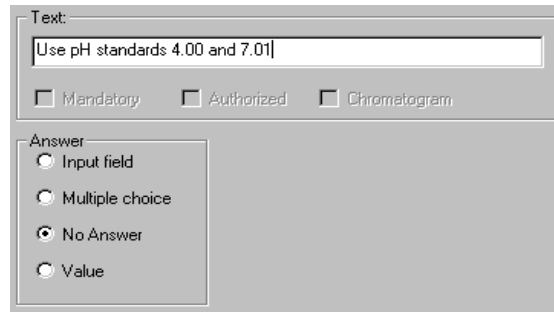


Figure 5-32. Options for no answer questions.

- **Value** accepts only numerical answers. Value questions may have specified maximum and minimum limits, and may be defined to accept only integer values.

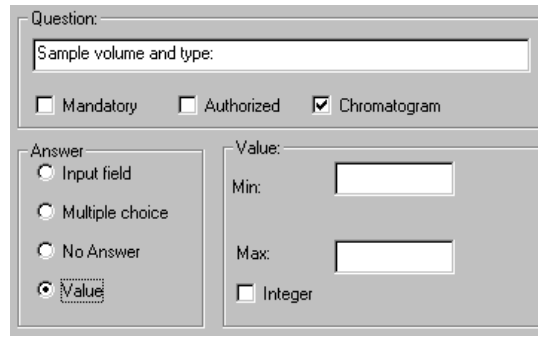


Figure 5-33. Options for value questions.

Press the **Preview** button to display the questions as they will appear when the method is run. (Alternative answers to multiple choice questions are not shown in this mode). From preview mode, press **Edit** to return to question editing mode.

Inserting a question

To insert a new question after an existing question:

1. Click on the existing question in the questions list.

2. Enter the text, status, type and answer for the new question as required. The **Answer** panel depends on the type of question:

   - Input field questions: Enter a default answer if required.
   - Multiple choice questions: Click on the input field under **Alternatives**, enter the answer and click on **Add/Delete**. Repeat this procedure to add other alternatives. New alternatives are always added at the end of the list. To remove an alternative, mark the alternative in the scroll list and click on **Add/Delete**.
   - Value questions: Enter maximum and minimum limits. Check the **Integer** box to if the question is to accept only whole numbers as answers.

3. Click on **Insert** to add the new question to the list.

   If the list is empty, the **Insert** operation creates the first question in the list.

Editing an existing question

To change the definition of an existing question, select the question to be changed. Change the text, status, type and answer as required and click on **Replace**.

Deleting a question

To remove a question, select the question and click on **Delete**. To remove all questions, click on **Delete All**.

5.6.4 Gradient

The gradient page provides a graphical overview of the block structure and eluent gradient in the current method. For scouting runs, click on the **Run** button to see the appropriate gradient for each run.

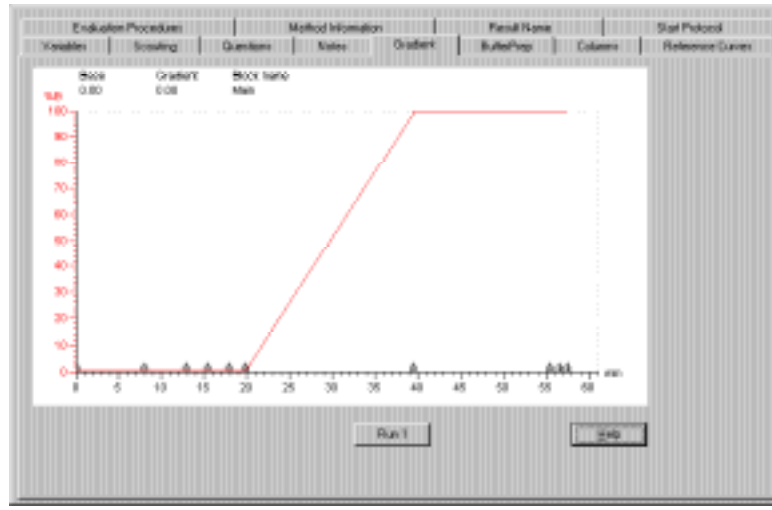


Figure 5-34. The Method editor gradient window.

Vertical cursor line

A vertical cursor line can be dragged from the y-axis with the mouse. As you drag the cursor line the current position is identified at the top of the page in terms of the block name, x-position in the currently displayed base and eluent concentration in % eluent B. The duration of variable length blocks are based on the default variable values.

The zoom function

To zoom in on a selected region of the Gradient page:

1. Press down and hold the left mouse button and drag a rectangle out on the screen to encompass the area to be viewed.

2. Release the mouse button. The display is now zoomed in on the selected area.

3. Repeat the process for further magnification of selected areas.

You can reduce the scale of the zoom in function in two ways, either:

- reverse each zoom in action a step at a time by displaying the right mouse button menu and selecting **Undo Zoom**, or,
- reverse all of the zoom in actions to the default scale setting by displaying the right mouse button menu and selecting **Reset Zoom**.

Changing the base

You can select the base shown on the x-axis, either time, volume or column volumes. Make the appropriate selection from the right mouse button menu option **Base type**. Changing the base for the display does not affect the base in the method instructions. You can therefore check how long a method will take simply by setting the axis scale to time, even if the method blocks are written in volume or column volume base.

Viewing hatch marks

You can display a grid in the Gradient page by selecting **Hatch** from the right mouse button menu.

5.6.5 Notes

Notes are descriptive comments that form part of the method documentation. There are four separate notes fields for method editing, start-up, run and evaluation respectively. Only the method notes can be edited from the Method editor: the other notes are accessible at the respective stages in a run.

To view the method notes, open the Notes page in the Run setup. Method templates are supplied with notes describing the system requirements for running the method. Read through these notes carefully before using a method. Click on the maximize notes button to expand a notes field to fill the notes page. Click on the same button again to restore the default display with all four notes fields visible.

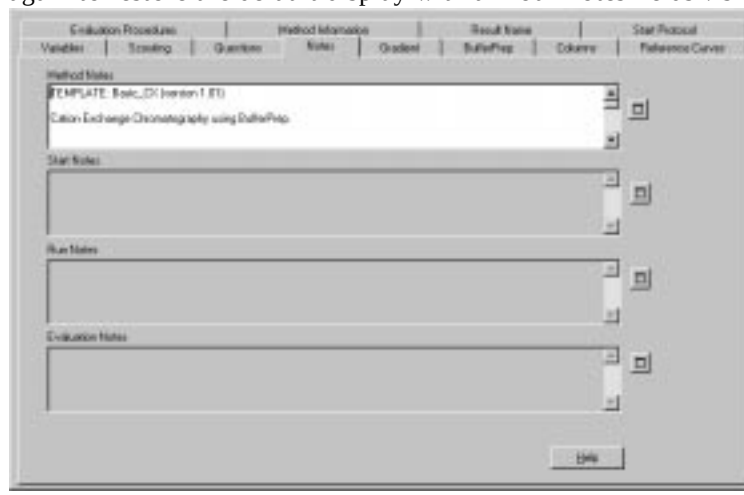


Figure 5-35. The Notes page in Run setup.

The notes are entered as free text and may be edited using standard Window editing functions to edit the notes. Words wrap automatically at the end of the field.

<Ctrl+X>	Cuts the marked text, saving it to the clipboard.
<Ctrl+C>	Copies the marked text to the clipboard.
<Ctrl+V>	Pastes the contents of the clipboard at the cursor.
<Delete>	Clears the marked text without saving it in the clipboard, or deletes the character to the right of the cursor if no text is marked.
<Backspace>	Clears the marked text without saving it in the clipboard, or deletes the character to the left of the cursor if no text is marked.

In the default method templates supplied with UNICORN, the method notes describe the system setup required by the method (e.g. eluent and sample inlets, outlets, column connections and so on). We recommend that you use method notes for this purpose in your own methods, to provide documentation of the method requirements. Bear in mind that method notes are saved with the method and apply to all runs made with the method. Use the start or run notes for run-specific information. The date and time when the method was created and last edited are saved automatically in the method information, and need not be entered in the method notes.

5.6.6 Evaluation procedures

Evaluation procedures can be called automatically at the end of a method to evaluate and/or print the results. Method templates supplied with UNICORN include procedures named:

Integrate_and_Print which integrates the first UV curve in the chromatogram and prints out the results

Print_Chromatogram which prints the chromatogram from the run with the scouting variables printed at the top.

User-defined procedures are created in the evaluation module and may be saved in method files (see Section 10.3). Procedures saved with one method file can be imported to another.

Note 1: A procedure in a method will not be updated when a procedure with the same name is changed in Evaluation. The same applies to report formats saved in a procedure.

Note 2: If you use an evaluation procedure to print results automatically from a run controlled from a remote station in a network installation, the results will be printed on the printer currently set up on the local station, not on the remote station. If however you execute the procedure interactively from the evaluation module on the remote station, the results will be printed on the printer set up on the remote station where you are working.

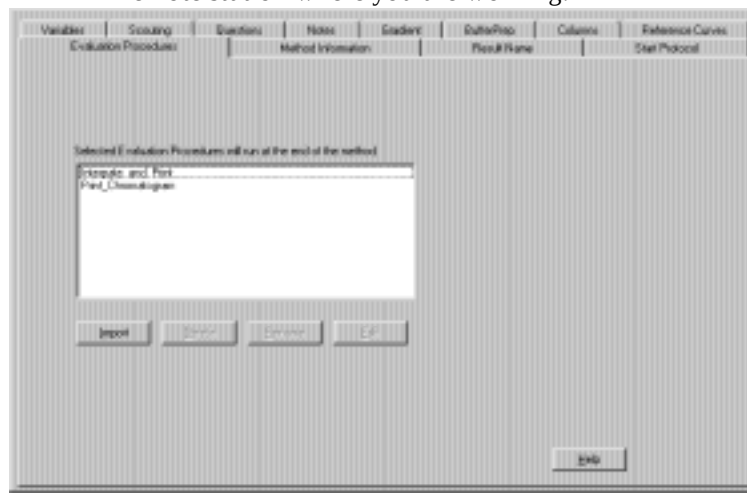


Figure 5-36. The Evaluation Procedures page in Run setup.

Defining and viewing procedures

Evaluation procedures are normally defined in the Evaluation module. Procedures imported to a method can also be viewed and edited in the Method editor; select the required procedure in the list and click on **Edit**. See Section 10.3.2 for a description of how to edit evaluation procedures.

Note: Evaluation procedures which process chromatogram data rely on consistent identification of curves in the result file for correct operation. If you include evaluation procedures with a method, make sure that references to curves in the procedure will be valid when the procedure is executed at the end of the run. See Section 10.3 for more details.

Selecting procedures to run

The Evaluation Procedures page lists all evaluation procedures associated with the method. Click on the procedure(s) which are to be executed at the end of the run. The procedures will be executed in the order they appear in the list.

Importing procedures

To import an evaluation procedure:

1. Select the Evaluation Procedures page and click on **Import.**

2. Choose a procedure from the **Select list**. You can also choose to import a procedure from another method. Select a method to show the procedures stored in the method. If you have chosen a method, click on **Evaluation Procedures** to return to the complete list.

3. If desired, change the procedure name in the **Import as** field.

4. Click on **Import**.

   The dialogue remains open until you click on **Close**, so that you can continue to add procedures from the same or different method files.

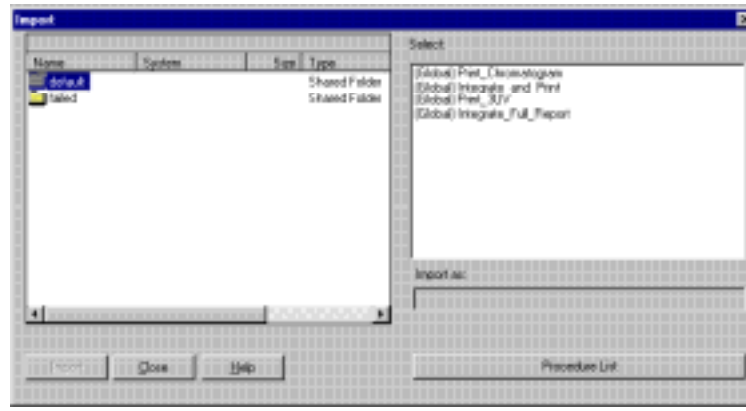


Figure 5-37. The Import procedures dialogue.

Deleting procedures

To remove one or more procedures from the method:

1. Select the Evaluation Procedures page and select the procedure(s) to be deleted.

2. Click on **Delete** and confirm the deletion.

Note: Procedures that you delete from the method are removed from the method file when you save the method.

Renaming procedures

To rename a procedure in a method:

1. Select the Evaluation Procedures page and click on **Rename**.

2. Select a procedure from the list and change the name in the **Rename item to** field.

3. Click on **Rename**.

The dialogue remains open until you click on **Close**, so that you can rename more than one procedure without closing the dialogue.

Editing procedures

To edit a procedure in a method:

1. Select a procedure on the Evaluation Procedures page and click on **Edit**.

2. Edit the procedure as described in Section 10.3.

3. Choose **File:Exit** from the procedure editor menu. (**File:Save** is not available in the procedure editor window when you edit procedures in a method. Changes are saved automatically when you close the procedure editor).

Note: Report formats in procedures cannot be edited or viewed.

5.6.7 Reference curves

Reference curves are curves from existing result files which you can display in the curve window of System control during a run. You can include up to 5 reference curves in a method. You choose which curves to display during the run with the **View:Curve contents** command in System control (see Section 6.2). Reference curves are only displayed during the run and the y-axis can be made active for the reference curve by clicking on its legend. Reference curves are not saved in the result file.



Figure 5-38. The Reference Curve page in Run setup.

Adding curves

To add a reference curve from a result file:

1. Select the Reference Curves page and click on **Import**.

2. Choose the result file containing the curve to be added and select the curve from the **Select** list.

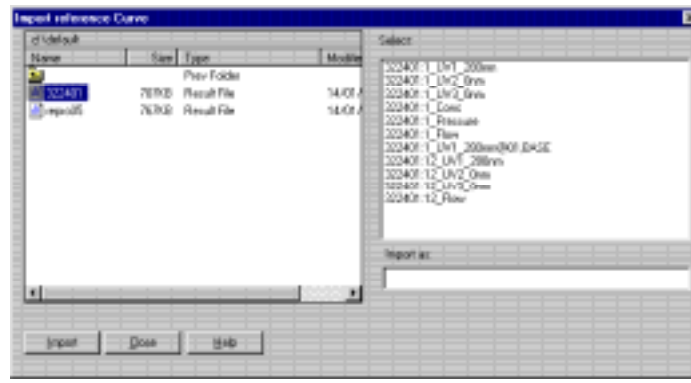


Figure 5-39. The Import Reference Curve dialogue.

3. If desired, change the curve name in the **Import as** field.

4. Click on **Import**.

The dialogue remains open until you click on **Close**, so that you can continue to add curves from the same or different result files.

Deleting curves

To remove one or more reference curves from the method:

1. Select the Reference Curves page and select curve(s) from the list.

2. Click on **Delete** and confirm the action.

Note: Deleting curves from the method does not affect the curves in the result file from which they were imported.

Renaming curves

To rename a reference curve in a method:

1. Select the Reference Curves page and click on **Rename**.

2. Select a curve from the list and change the name in the **Rename item to** field.

3. Click on **Rename**.

The dialogue remains open until you click on **Close**, so that you can rename more than one curve without closing the dialogue.

5.6.8 Columns

You can look at the parameters of the column selected for your method by clicking on it in the Column page. The column parameters will be displayed in the **Column Data** field. If you are performing scouting runs with different columns, all of these will be listed. Select the appropriate column to display the parameters.

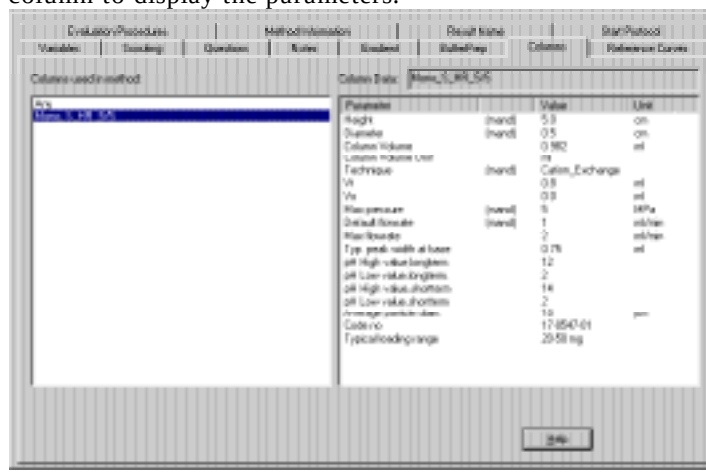


Figure 5-40. The Columns page in Run setup.

5.6.9 BufferPrep

BufferPrep is only available for some ÄKTAdesign systems. BufferPrep allows a buffer of any pH and salt concentration to be prepared on-line from 4 stock solutions. This removes the need to manually prepare new buffers every time the pH needs to be changed. Linear and step salt gradients can be run and pH can be used as a variable scouting parameter. BufferPrep is optimised for cation and anion exchange chromatography. It can be used for gel filtration, but not for other chromatographic techniques. For a complete description of BufferPrep, see the User Manual for ÄKTAdesign systems.



Figure 5-41. The BufferPrep page in Run setup.

To use BufferPrep you must select a template that ends with either 'ax' or 'cx'.

Note: If a suitable template is not available you can create a BufferPrep method by selecting the BufferPrep page, clicking on the **BufferPrep ON** radio button. The instruction **BufferPrep_pH** must be available at breakpoint zero at the beginning of the method. The method must not contain the instructions **PumpAInlet** or **PumpBInlet**.

1. On the BufferPrep page select a recipe from the pull-down list, either AIEX or CIEX, which are recipes covering a broad pH range. Otherwise select one of the single buffer recipes for more narrow pH ranges. All information relevant to the selected recipe will be displayed on the page.

2. Prepare the required stock solutions. The solutions and the inlets to which they should be connected are displayed to the right of the

page. Accuracy of preparation is essential. The four stock solutions consist of:

i. A mix of buffering components

There can be up to five different buffering components enabling a broad pH range to be covered.

ii. An acid (HCl) or base (NaOH) for pH on-line titration.

iii. Distilled water.

iv. An inert salt (e.g. NaCl) for salt gradient formation.

3. Select the Variables page. Set the required pH for the run in the variable **BufferPrep_pH**.

Alternatively, if you want to perform pH scouting, click on the Scouting page and select **BufferPrep_pH** as a scouting variable and enter the pH values for the different runs.

The recipe saved in the method (the one selected on the BufferPrep page) cannot be edited although fine tuning is possible (see below). However, the recipes in the list of all BufferPrep recipes (**File:BufferPrep recipes**), can be edited (see section 5.10). New recipes can also be created (see Section 5.10).

Fine tuning with correction factors

In order to obtain high pH accuracy, the recipe can be fine tuned around a specific pH by setting correction factors. Run BufferPrep manually at 0% and 100% and change correction factors in the method as follows:

1. Select the **Recipe** under group **Other** in **System Control:Manual**. Click on **Execute**. Set the pH in the instruction **BufferPrep_pH** in group **Pump**. Click on **Execute**. Set the flow rate to be used during the run in instruction **Flow**. Click on **Execute**.

2. Check the pH reading when stable. Allow at least 30 ml of eluent to pass through before expecting a steady pH reading.

3. Change to 100% B by setting the **Gradient** instruction in **Manual:Pump** to 100% for **Target** and 0 for **Length**. Click on Execute.

4. Check the pH reading when stable at 100% B.

5. If the readings are acceptable at both 0% and 100%, the correction factors do not need to be changed.

6. If the readings are not acceptable, click on **Corr. Factors** in the BufferPrep page in the method.

7. Enter the deviation at 0% and 100%. (Example: if the pH is set to 7.0 and the actual pH is 7.1 enter 0.1, if the actual pH is 6.9 enter -0.1).

Note: If correction factors already exist, the measured pH deviation should be added to the old factors.

8. Save the method.

Note: When changing the correction factors for the recipe selected in the method, the recipe with the same name in the list of all BufferPrep recipes is not affected. The changes will only apply in the specific method.

For changing the default correction factors in a recipe not saved in a method, see Section 5.10.2.

5.6.10 Method Information

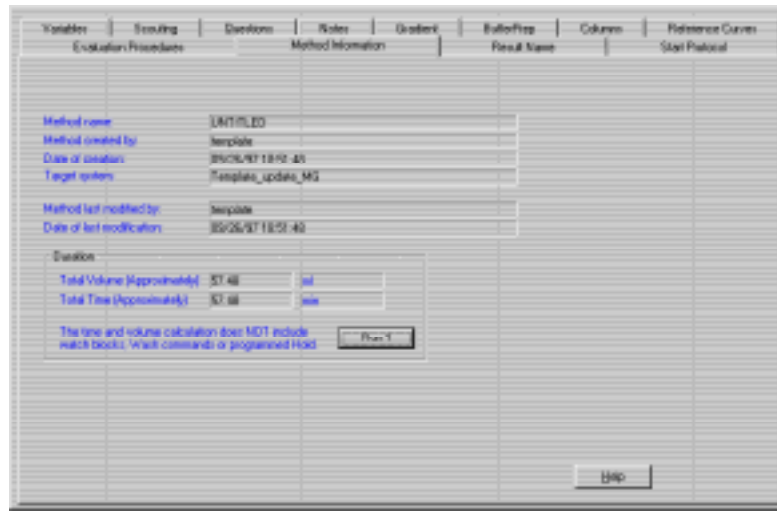


Figure 5-42. The Method Information page in Run setup.

The Method Information page displays information about the method, such as method name, target system for creation and date of last

change, estimated eluent consumption and duration in time of the method. These figures for the latter two are based on values for methods with variable length parameters, and will be changed if the values are changed. If the method includes a scouting scheme, click on the **Run 'x'** button under Scouting to see the estimated times and volumes for the different scouting runs.

The Method Information page is for information only and cannot be edited.

5.6.11 Result Name

Use the Result Name page to specify how the result files will be named for the results of a run, and where the result file will be saved. You can also specify the name of the special scouting folder where results from scouting runs will be stored.

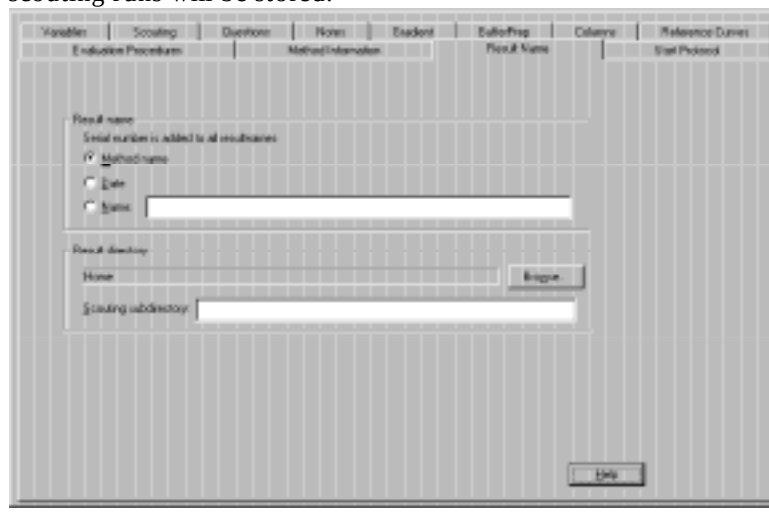


Figure 5-43. The Result Name page in Run setup.

The result file name is constructed by adding a 2-digit serial number to one of the base options listed below. The serial number is incremented automatically each time the method is run.

- the method name plus a 2-digit serial number
- the date of the run (in a 6-digit format determined by the country setting in Windows NT) plus a 2-digit serial number
- a freely specified name (within the file naming restrictions in the operating system) plus a 2-digit serial number

If the result file folder already contains files with the same file name base, the serial number is incremented automatically. For scouting

runs, the 2-digit serial number will be the number of the executed run column in the scouting scheme.

By default, result files are stored in the home folder of the user who starts the run. To change the folder where the result file will be stored, press the **Browse** button, double-click on the required folder icon and press **Close**.

Note: The result name may be specified as changeable in the Start protocol (see Section 5.6.10). In that case, the specification in the Result page serves to generate the suggested result name, which may be changed at the start of the run.

Scouting results will be saved in a special folder on the result file path. Enter a name for the folder in the **Scouting folder** field. Each time the scouting method is run, a new folder will be created with the name and a serial number (e.g. entering IEXSC will create folders IEXSC 1, IEXSC 2 etc.).

5.6.12 Start protocol

The Start protocol determines which items of the Run setup are displayed (and may in appropriate cases be altered) at the start of a run. Open the Start Protocol page and check the items that are to be displayed.



Figure 5-44. The Start Protocol page in Run setup.

Variables

If this box is checked, values for method variables will be displayed and can be changed at the start of the run. These values will override the default values for the particular run, and will be saved in the result file. The default values stored in the method are however not affected. Variables defined for scouting cannot be changed in the Variables page.

If the **Variables** box is not checked, the run will be executed with default values for all variables as defined in the method, unless scouting is used (see below).

Scouting

If this box is checked, the scouting scheme will be displayed and can be changed at the start of the run. Changes will override the default settings and values for the particular run, and will be saved in the result file.

Note: Variable values in the scouting scheme will override any values in the Variables page.

If the **Scouting** box is not checked, scouting settings saved with the method will apply when the method is run.

Text Method

Displays the method instructions. Double-click on Block instruction, as denoted by the blue square and '+' mark, to display or hide the instructions in the called block (see Section 5.4.1). Method instructions cannot be changed from this display.

Questions

If this box is checked, questions defined in the method will be displayed at the start of the run.

Important!
If the **Questions** box is not checked, questions will not be displayed even if they are defined as mandatory. Since the answers to questions can form an important part of the run documentation, you are recommended always to check the **Questions** box.

Notes

If this box is checked, the notes page will be displayed at the start of the run. You can enter notes in the **Start notes** field but not in any of the other fields. You can use the scroll bar if necessary to read notes in the **Method notes** field.

The start of the run is the only occasion when you can enter start notes. If the **Notes** box is not checked, the notes will not be displayed and you cannot enter start notes for the run.

Gradient

If this box is checked, you can view the gradient at the start of the run.

BufferPrep

If this box is checked the recipe selected in the method will be displayed at the start of the run. You cannot change recipe at the start of a run. Only available to some systems.

Columns

If this box is checked, you can view the available column definitions (see Section 5.6.7). The column definition used in the method run is selected with the Base instruction, and may be changed at the beginning of the run on the Variables page if the columns parameter is defined as a variable (see Section 5.6.1).

Reference curves

If this box is checked, the reference curves associated with the method will be displayed at the start of the run. You can add, delete and rename curves at the start of the method. All curves in the list can be displayed in System control during the run.

If the **Reference curves** box is not checked, the curve settings saved in the method will apply.

Evaluation procedures

If this box is checked, the evaluation procedures set to execute at the end of the method will be displayed at the start of the run. You can change the choice of procedures to execute, but you cannot add or remove procedures. (Procedures are stored as part of the method file, which cannot be changed at the start of the run).

If the **Evaluation Procedures** box is not checked, the procedure settings saved in the method will apply at the end of the method.

Method information

If this box is checked, the method information (including creator, target system, and date and time of creation and latest change) will be displayed at the start of the run. You cannot edit the method information.

Settings

If this box is checked, the settings (including alarms, monitors and curve configuration) will be displayed for information at the start of the run.

To change settings, use the **System:Settings** command in System control before starting the run (see Chapter 15).

Calibration

If this box is checked, the monitor calibration settings will be displayed at the start of the run.

If the **Calibration** box is not checked, you can still calibrate the monitors before the run is started by using the **System:Calibrate** command in System control.

Result name changeable

If this box is checked, you can change the result name when the run is started. Click on the **Browse** button to change the result folder.

If the **Result name changeable** box is not checked, the result name will still be displayed, but neither the name nor the folder can be changed.

Scouting start protocol

Check the appropriate option for Scouting start protocol display in scouting runs. If you check **First run only**, parameters for the scouting runs can be adjusted at the beginning of the first run only, and the runs will thereafter be performed automatically without operator intervention. If you check **All runs**, the Scouting start protocol will be displayed at the beginning of each run in the scouting scheme, allowing the operator to adjust variable values and exclude or include runs as required.

5.7 Saving the method

5.7.1 Saving a method

Click on the **Save** button on the toolbar or choose **File:Save** to save the method. The method remains open in the Method editor when it has been saved, so that you can continue editing if you wish. Once the method has been saved, choosing **File:Save** saves the current state of the method under the same name. If you want to save a copy of the method under a new name, choose **File:Save As** and enter the details as described below.

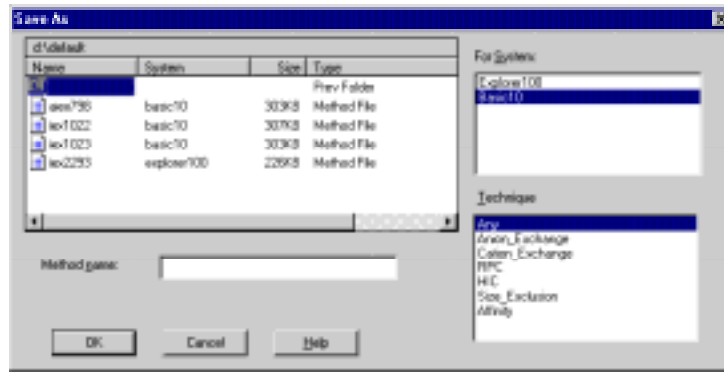


Figure 5-45. Save As dialogue for saving a method.

1. Enter a name for the method. Method names may contain letters (A-Z) and digits (0-9). The case of letters is not significant. The method name must be unique for the chosen system within the folder (see steps 2 and 3 below).

2. By default, the method will be saved in your home folder. To change the folder, double-click on the appropriate folder icon in the Methods panel.

3. If you have more than one system available, choose the system for which the method is intended. The method can only be run on the system for which it is saved. Remember that different systems may have different configurations and control capabilities.

Note: Each method is written for a specific strategy. The function of the method cannot be guaranteed on systems having other strategies.

4. Click on **OK**.

5.7.2 Saving as a template

You can save the method as a template if you have **Edit global lists** authorisation.

1. Choose **File:Save as Template**. The **Save As Template** dialogue is displayed.

2. Enter a name for the template in the **Name** field, or choose an existing template name from the list. If you choose an existing name your will overwrite the existing template.

3. Choose the system for which the template is intended in the **For system** field.

4. Select the **Technique** from the lists as appropriate.

5. Click on **OK**.

The templates for each system are common for all users. Be restrictive in saving methods as templates. We recommend that only methods that are useful for all users are saved as templates.

5.7.3 Deleting a template

You can delete templates if you have **Edit global lists** authorisation.

1. Choose **File:Delete template**.

2. Select the system and the template to delete, and click on **Delete**.

3. Confirm the action.

Note: The templates for each system are common for all users. Be restrictive in deleting templates.

5.8 How to use selected method instructions

This section provides recommendations for how to use some common programming features in UNICORN methods.

5.8.1 Base instruction

Every method block must start with a Base instruction, defining the base for calculating breakpoints. The base may be volume (ml or l depending on the scale defined in the system strategy), time (min), column volume CV (defined as a numerical value or taken from the column definition). For all blocks other than the main block, the base

may also be defined as SameAsMain, which means that the block will inherit the base defined in the main block. Different blocks may use different bases.

Use the base which most closely suits the purpose of the block. Column volume is recommended as the base for most steps in a run. In some situations, it may be more suitable to use time or volume base for individual blocks.

Be careful when changing the base for an existing method. Changing between time and volume base can affect the relative duration of steps in the method if different steps use different flow rates.

Note: For method blocks which use a volume or column volume base, make sure that the flow rate is not zero. Volume breakpoints are calculated from the flow rate of the pump, and the method will not progress if the flow rate is zero.

The parameters for the Base instruction differ slightly according to whether a named column definition is used:

Column parameter: Any

If the **Column** parameter in the Base instruction is set to **Any** and the **Base** parameter is set to **CV**, the column volume is set numerically by the **Volume** parameter. The column volume may be defined as a variable, allowing the scale of the run to be decided when the method is actually run.

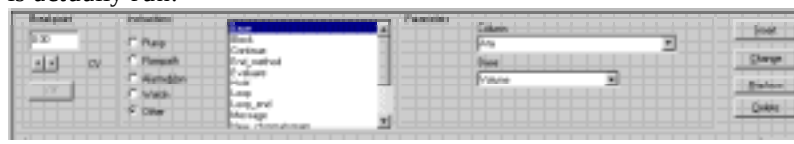


Figure 5-46. With column volume selected for the Base instruction and Any selected for the Column parameter, the column volume is entered numerically and may be defined as a variable.

Column parameter: named column

If a named column is selected for the **Column** parameter in the BASE instruction, the volume specified in the selected column definition will automatically be used for column volume in the method block. The column volume for base CV cannot then be changed in the instruction or defined as a variable. The Column parameter can however be defined as a variable (recommended). Choosing a column definition also enables linear flow rate and column performance calculations (see Sections 5.8.6 and 10.1 respectively).

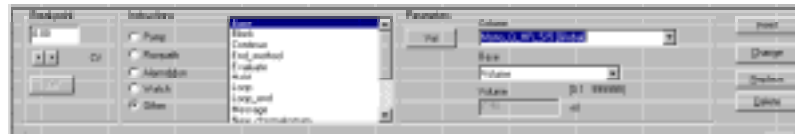


Figure 5-47. Using a named column definition in the Base instruction. The column volume is taken from the column definition and cannot be changed in the instruction. The column name can be defined as a variable.

In cases where a template and column are chosen, it is easy to select other columns for that template in the Variables page. To select a column for a method when the method is not selected from a template:

1. Define new columns in the **Columns** list if required (Section 5.9).

2. In text instruction mode, mark the Base instruction in the method (the first instruction).

3. For the **Column** parameter in the instruction box, choose the required column from the pull-down list.

4. Define the **Column** parameter as a variable (recommended). This will make it easy to change the column selection for different runs (see Chapter 4 for defining variables).

5. Click on **Change** in the text instruction box.

Note 1: A selected column definition applies locally within the block for which it is selected, and is not transferred to other blocks. We strongly recommend that the column definition is selected for the main block, and that the base for other blocks is set to SameAsMain. This ensures that other blocks will inherit the column definition values from the main block.

Note 2: A column definition can be chosen and defined as a variable even if the base for the block is set to volume or time. Parameters in the column definition will then be used for linear flow rate and column performance calculations.

Note 3: If you want parameters to be updated when you change the column, you must define flow, pressure and averaging time as variables.

The Methodbase instruction

Volume or column volume base is calculated from the flow rate of the

GradientPump (AB) or the sample pump, selected with the instructions Methodbase. If no Methodbase instruction is included in the method, the default setting GradientPump will be used. The default setting can be changed in **System:Settings**.

5.8.2 Instructions at the same breakpoint

Instructions placed at the same breakpoint in a block are executed simultaneously, with the exception of successive Block instructions which are executed in the sequence in which they are written. This can have important consequences in some situations. For example, the instruction sequence

```
...
0.00  Block WASH
0.00  FractionCollector 5{ml}
0.00  Block ELUTE
...
```

will start the fraction collector when the Wash block is completed.

5.8.3 Controlling block and method length

The length of a block is determined by the breakpoint of the last instruction in the block. A block in which all breakpoints are at 0 will take no time or volume during a run, e.g.

```
(BASIC_START_CONDITIONS_IEX)
0.00    Base SameAsMain
0.00    ColumnPosition (Position1Bypass)#Column_position
0.00    AveragingTime (2.56)#UV_Averaging_time {sec}
0.00    Wavelength 1, (280)#Wavelength_1 {nm}, 254 {nm}, 215 {nm}
0.00    Alarm_Pressure Enabled, (5)#Pressure_limit {MPa}, 0.00 {MPa}
0.00    BufferValveA A11
0.00    Gradient (0.00)#Start_ConcB {%B}, 0.00 {base}
0.00    BufferPrep_pH (0)#BufferPrep_pH
0.00    Flow (1)#Flow_rate {ml/min}
0.00    AutoZeroUV
0.00    End_block
```

To extend the length of a block without performing any other operation, set the breakpoint of the End_Block instruction appropriately, e.g.

```
(Equilibration)
0.00    Base SameAsMain
4.00    End_block
```

During a run, the overall time or volume is determined by the sum of the block lengths. Note that the length of the main block does *not* indicate the overall length of the method (the main block often consists only of calls to other blocks and has zero length). The method length can be checked in the Gradient window of System control.

Accumulated time/vol	*Block time/vol*	*Instruction*	
		(Main)	
0	0	0.00	Block EQUILIBRATE (Equilibrate)
0	0	0.00	Base SameAsMain
4	4	4.00	End_block
4	0	0.00	Block SAMPLE_INJECT(Sample_inject)
4	0	0.00	Base SameAsMain
4	0	...	
9	5	5.00	End_block
9	0	0.00	Block GRADIENT
		(Gradient)	
9	0	0.00	Base SameAsMain
9	0	0.00	Gradient 100,20
29	20	20.00	End_block

Table illustrating the relationship between accumulated and block time/ volume for a simplified method fragment.

Depending on how conditional calls are used (see Section 5.8.8), the overall method time or volume may vary according to watch events during the run.

5.8.4 Messages

Use messages to inform the operator of the progress of the run. It is a good idea to issue messages at critical points in the method, e.g. when Watch instructions are used for conditional events or at the end of a gradient (see below). The example block below starts the fraction collector and informs the operator:

```
(Start_frac)
0.00  Base SameAsMain
0.00  Message "Fraction collection
      started",Screen
0.00  FractionCollector 5{ml}
0.00  End_block
```

Messages which are set to Screen will be displayed on the screen during a run, and will remain there until acknowledged by the operator. Messages can also be set to Noscreen: these will be recorded in the run log but not displayed on the screen.

5.8.5 Pausing a method

A method can be programmed to pause at critical points. There are two instructions for this purpose:

Hold Suspends execution of the method, but continues to pump eluent at the current flow rate and concentration settings. This instruction is useful e.g. for giving the operator time to load a sample loop:

```
(Sample_injection)
0.00  Base SameAsMain
0.00  Message "Load the sample loop with sample
      then press Continue", Screen
0.00  Hold
0.10  InjectionValve Inject
5.00  InjectionValve Load
5.00  End_block
```

Pause Suspends execution of the method and stops the pumps so that the system comes to a standstill. In BioProcess System, valves are also set to their default position. In ÄKTAdesign systems and BioPilot System valves remain in the position they were in before the pause. The pause may be defined as indefinite or for a given number of minutes. This instruction is most useful for stopping the system in the event of an unexpected condition.

In both cases, the method may be resumed by pressing the **Continue** button in the System control toolbar (see Section 6.2).

5.8.6 Linear flow rates

Linear flow rates (cm/h) can be specified for Flow instructions if a column is selected on the Variables page or for the Base instruction in the block (see Section 5.8.1). The volume flow rate is calculated from the specified linear flow rate and the column diameter as given in the column definition. To use a linear flow rate, check the **Linear Flow** option in the instruction dialogue for the Flow instruction. The calculated volumetric flow rate is shown during runs.

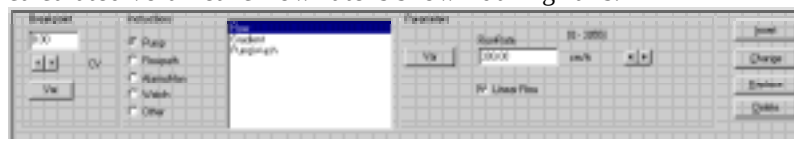


Figure 5-48. Setting linear flow rate in a method block. The linear flow rate option is only available if the base is set in terms of a named column definition.

Note: If a column is not selected in the Base instruction, linear flow will not be available.

5.8.7 Eluent concentrations and gradients

Both gradients and immediate changes in eluent concentration are defined in UNICORN methods with the single Gradient instruction.

The Gradient instruction takes two parameters:

Target which is the final eluent composition expressed in % eluent B

Length which is the duration of the gradient (not the breakpoint at the end of the gradient).

The instruction may be read as "form a Gradient to reach Target after Length". The starting point for the gradient is always the current eluent composition. For example:

```
10.00 Gradient 50{%B}, 20{base}
```

forms a gradient to 50% B starting at breakpoint 10 with duration 20 method base units (i.e. finishing at breakpoint 30). If the current eluent concentration is greater than 50% B, the gradient will be negative.

Forming a step gradient

A step gradient is an immediate change in eluent composition. To form a step gradient in UNICORN, set the **Length** parameter to 0 in the Gradient instruction.

For example:

```
10.00 Gradient 50{%B}, 0{base}
```

forms a step from the current eluent composition to 50% B at breakpoint 10. The method continues with 50% B.

Note: For BioPilot System, or BioProcess System, do not place two gradient instructions at the same breakpoint (e.g. for first stepping to a new eluent concentration and immediately starting a linear gradient). Only the last gradient instruction at a given breakpoint will be executed. Separate the instructions with a short interval instead, for example:

```
10.00 Gradient 50{%B}, 0{base}
10.10 Gradient 90{%B}, 30{base}
```

Setting the initial eluent composition

To set the initial eluent composition, set a step gradient to the required concentration at breakpoint 0 at the beginning of the method. This instruction can suitably be placed in the Main block, e.g. to start at 50% B:

```
(Main)
0.00  Base CV, (10)#Column_volume
0.00  Gradient 50{%B}, 0{base}
...
```

Breakpoints during gradients

The breakpoint for a Gradient instruction defines the time or volume (according to method base) for the start of the gradient. A gradient with a non-zero duration occupies time and volume in the method, and breakpoints for other instructions may be set to occur before the gradient is completed. For most instructions, the instruction is simply carried out at the requested breakpoint, while the gradient is forming. The following instructions affect the gradient, however:

- a new Gradient instruction will start a new gradient at the requested breakpoint. Any remaining duration of the previous gradient is ignored.
- a new Flow instruction will change the eluent flow rate at the requested breakpoint. If the current base is volume or column volume, the duration of the gradient will be changed. If the method base is time, the volume of the gradient will be changed.
- an End_Method instruction will stop the whole method, interrupting the gradient.

If an End_Block instruction is encountered during a gradient, the gradient formation will continue uninterrupted unless a new Gradient instruction is issued in the next block. This means for example that a block can be called conditionally during gradient formation without interrupting the gradient.

Gradients with variable duration

For many purposes, it can be useful to define the length of the gradient as a variable. When this is done, breakpoints for instructions issued during or after the gradient in the same block are automatically shifted in proportion to the length of the gradient, with the same functionality as **Change** in the text instruction editor (see Section 5.4.4).

It can often be useful to include a MESSAGE instruction at the end of a gradient to inform the operator that the gradient is completed. This can also help to make the method easier to follow in the Method editor by marking the end of the gradient. For example:

```
...
10.00 Gradient 50{%B},
      (20)#Gradient_duration{base}
30.00 Message "Gradient completed", Screen
...
```

The default gradient duration is 20 method base units, but different values can be set by changing the variable value in the Run setup. The message will always appear at the end of the gradient. For example, if the duration is set to 40 by changing the variable value, the message will appear at 50. The same adjustment is made if the default gradient length is changed by altering the **Length** parameter in the instruction box.

Breakpoints during a gradient are adjusted in proportion to the actual gradient length. In the following example, both messages will always appear at the right time - if the gradient duration is set to 40 method base units, the first message will appear at 30 units and the second at 50:

```
...
10.00 Gradient
      50{%B},(10)#Gradient_duration{base}
15.00 Message "Gradient 50% completed", Screen
20.00 Message "Gradient completed", Screen
...
```

Note: Gradient functions over time and any instruction that you want to insert after a gradient should be placed after the combined breakpoint and gradient length.

5.8.8 Conditional instructions

Conditional (Watch) instructions allow the progress of a run to be determined by the events during the run, e.g. start collecting fractions when the first peak elutes, or equilibrate the column until the eluent conductivity has reached a given value. The system strategy includes a Watch instruction for each monitor defined in the system. A watch is active from the point at which it is issued until either

- the watch condition is met
- a new watch is set for the same monitor
- a Watch_Off instruction is issued for the monitor

Watch conditions

The conditions for which a watch can be set are as follows for most monitors:

Greater_than	The signal exceeds a specified value.
Less_than	The signal falls below a specified value.
Slope_greater_than	The rate of change of the signal exceeds a specified value, expressed in monitor units/ minute (e.g. mAU/min).
Slope_less_than	The rate of change of the signal falls below a specified value, expressed in monitor units/minute (e.g. mAU/min).
Less_than_or_valley	The signal falls below a specified value or a valley is detected. A valley is detected only after a peak_maximum has been detected, and the valley is defined by a local minimum followed by an increase to 102 % of the local minimum value plus the Delta_Peak value (see below).
Peak_max	The signal falls to a specified fraction of the most recent peak maximum minus the Delta_Peak value (see below). Factor=1 detects peak maximum.

Stable_baseline — The signal is stable within the limits of the Delta_Base value (see below) for the period specified by the minutes parameter.

To determine suitable values for watch conditions, it is often most convenient to examine data from a test run. For slope values, use the **Differentiate** function in the evaluation module to measure the slope of the test chromatogram.

Note: The slope criteria operate on the arithmetic value of the slope, so that a value of -3 is less than a value of -2. The end of a peak is thus detected by Slope_greater_than with a negative value (the slope is negative but increasing).

Two conditions apply for air sensors, although may be handled differently dependent on the system:

Equal 0 — Air is not detected by the sensor.

Equal 1 — Air is detected by the sensor.

Note: To use the Watch_as instruction for air sensors, the AsAlarm setting in **Alarms&Mon** must be disabled (use the Method editor to disable the alarm locally in a method, or the settings to disable the alarm for all methods, see Section 15.1). The AsAlarm setting overrides any Watch_As instruction, and if the alarm is enabled the method will pause when air is detected.

Settings for watch conditions

The mode of function of the WatchPar“Monitor” instruction in ÄKTA design systems is affected by certain parameters within the instruction (see below).

Note: The instruction is called Peak"Monitor" for BioPilot and BioProcess, e.g. PeakUV

Delta_Peak

The Delta_Peak setting (called MinPeak for BioPilot and BioProcess) helps the software to detect valleys, peaks and Peak maximum, and to ignore noise in the chromatogram.

The Delta_Peak setting:

(a) sets the threshold for signal increase after a local minimum which will be interpreted as a valley for the Less_than_or_valley condition. A valley and a new peak are detected when the signal increases to 102% of the local minimum plus the Delta_Peak value. Note that a valley is detected only after a peak_maximum has been detected.

(b) sets the threshold for signal decrease after a local maximum which will activate the Peak_max condition. Peak_max is detected when the signal falls to the specified fraction of the most recent peak maximum minus the Delta_Peak value.

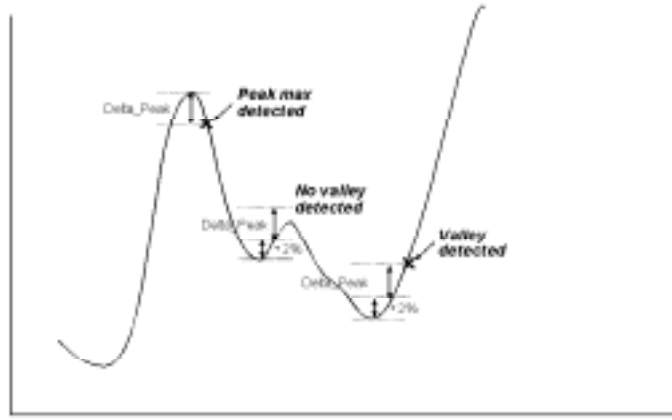


Figure 5-49. Illustration of the Delta_Peak setting. Peak max is detected when the signal falls by Delta_Peak from a local maximum if the Peak_max factor is set to 1.

The Delta_Peak value should be set large enough so that signal noise does not activate the conditions, and small enough so that the condition is activated close to the valley or peak. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Setting a value that is too high can prevent a new peak from being detected after a local minimum.

Delta_Base

Delta_Base (called D_Baseline for BioPilot and BioProcess) helps the software to determine when the baseline is considered to be stable, i.e. it defines the permitted variation for the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the watch instruction.

The Delta_Base setting affects only the Stable_baseline condition.

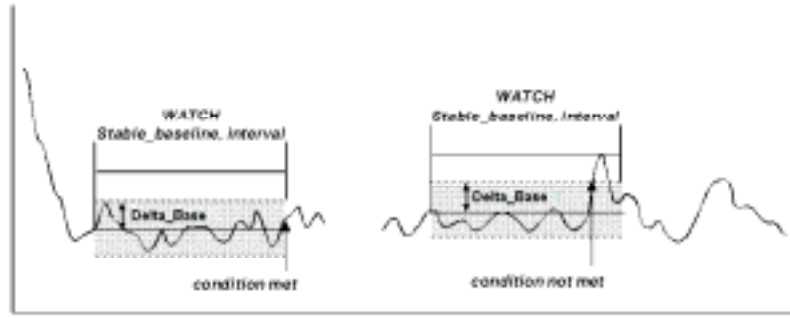


Figure 5-50. Illustration of the Delta_Base setting. The condition Watch Stable_baseline is met if the signal does not deviate by more than ±Delta_Base from baseline during the time interval specified for the watch. The baseline value is determined by the signal at the start of the watch. If the condition is not met, a new interval is started with a new baseline value defined by the signal level at the start of the new interval.

Permanent settings for Delta_Peak and Delta_Base are entered with the **WatchPar** instruction (e.g. **WatchPar_UV**, **WatchPar_Cond**) under **System:Settings** in the System control menu (Chapter 15). Temporary settings that apply only for the duration of a given run can be entered with the **WatchPar** in the **Alarms&Mon** group in the Method editor.

Watch actions

The following actions can be taken when a watch condition is met:

Block name	Call the named block.
Pause, Hold	Pause or hold the method (see Section 5.8.5)
Continue	Continue the method if paused or held
End_block	End the current block and return to the point from which the block was called
End_method	End the method
Ready	Indicates that the next step in a MethodQueue may start.

Watch example 1: Simple equilibration

In the following example:

The Watch is started on the conductivity signal and the method is then put on Hold. When the Watch_cond condition is fulfilled, Continue is issued and Watch_cond is turned off automatically. Method execution now continues issuing a Watch_UV command. Again the method is put into Hold until the Watch condition is fulfilled. This method works satisfactorily although one drawback is that it might never end, an thus consume all of the buffer if the conditions for some reason are unfulfilled. See Watch example 2 below.

```
(Main)
0.00  Base CV, 0.97{ml}, Resource_Q_1_ml
...
0.00  Block EQUILIBRATE
...
(Equilibrate)
0.00  Base SameAsMain
0.00  Watch_Cond, Less_than, 5 {mS/cm}, Continue
0.00  Hold
0.10  Watch_UV, Less_than, 100 {mAU}, Continue
0.10  Hold
0.20  End_block
```

Watch example 2: Equilibration with safeguard

Equilibrate the column until the conductivity is below 5 mS/cm. If this value is not reached within 6 column volumes, pause the method with a message. Equilibrate until the conductivity value is "stable" (allowed to vary by max ±2 mS/cm) over a period of at least 5 minutes. If this condition is not met within 10 column volumes, pause the method again. At each pause, the operator can decide whether to continue or abort the run.

```
(Main)
0.00  Base CV, Resource_Q_1_ml
...
0.00  Block EQUILIBRATE
...
```

```
(Equilibrate)
0.00  Base SameAsMain
0.00  Block COND_LESS_THAN
0.00  Block COND_STABLE
0.00  End_block
(Cond_less_than)
0.00  Base SameAsMain
0.00  Watch_Cond, Less_than, 5 {mS/cm},
      End_block
6.00  Message "Low conductivity not reached",
      Screen
6.00  Pause -1.00 {minutes}
6.10  End_block
(Cond_stable)
0.00  Base SameAsMain
0.00  WatchPar_Cond, 0.50 {mS/cm}, 2 {mS/cm}
0.00  Watch_Cond, Stable_baseline, 5 {minutes},
      End_block
10.00 Message "Conductivity not stable", Screen
10.00 Pause -1.00 {minutes}
10.10 End_block
```

Watch example 3: Safe sample injection

This example applies to systems that have air sensors. Inject 300 ml sample with pump C directly on to the column. If there is less than 300 ml sample so that air is detected in the inlet, switch to eluent for the remainder of the injection volume. The Watch_AS1 instruction sets a watch on the inlet air sensor.

```
(Main)
0.00  Base, CV, 1.0
...
0.00  Block Sample_Inject
...
(Sample_inject)
0.00  Base Volume
```

```
0.00  InletC C2
0.00  FlowC 10 {ml/min}
0.00  Methodbase C
0.00  Watch_AS1 Equal, 1, No_more_sample
300.00Methodbase AB
300.00FlowC 0 {ml/min}
300.00End_block
(No_more_sample)
0.00  Base Volume
0.00  InletC C1
0.00  End_block
(No_more_sample)
0.00  Base Volume
0.00  InletC C1
0.00 End_block
```

Watch example 4: Collecting absorbance peaks

Collect one or two absorbance peaks through outlets F3 and F4 respectively, allowing the rest of the effluent to go to waste. Each called block (except End_collect) resets the watch condition so that the method will react correctly to subsequent changes in the UV absorbance.

```
(Main)
0.00  Base CV, Resource_Q_1_ml
...
0.00  Block ELUTION
...
(Elution)
0.00  Base SameAsMain
0.00  Gradient 100 {%}, 20 {Base}
0.00  Watch_UV, Greater_than, 100 {mAU}, Peak_1
20.00 Message "End of gradient", Noscreen
20.00 End_block
(Peak_1)
```

```
0.00  Base SameAsMain
0.00  OutletValve F3
0.00  Watch_UV1, Less_than_or_valley, 100
      {mAU}, Waste
0.00  End_block
(Waste)
0.00  Base SameAsMain
0.00  OutletValve F1
0.00  Watch_UV1, Greater_than, 100 {mAU}, Peak_2
0.00  End_block
(Peak_2)
0.00  Base SameAsMain
0.00  OutletValve F4
0.00  Watch_UV1, Less_than, 100 {mAU},
      End_collect
0.00  End_block
(End_collect)
0.00  Base SameAsMain
0.00  OutletValve F1
0.00  End_block
```

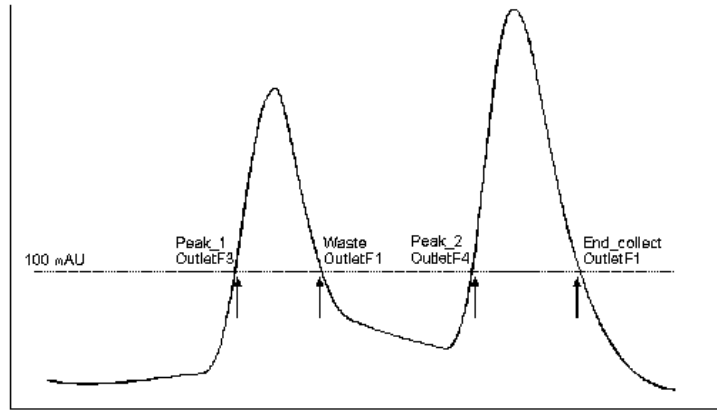
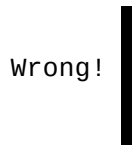


Figure 5-51. Peaks collected by the method in example 4 above.

The design of a method of this kind (with several WATCH instructions for the same monitor) is important. The following construction appears simpler but is incorrect:




```
...
0.00  Watch_UV, Greater_than, 100 {mAU}, Peak_2
0.00  Watch_UV, Less_than, 100 {mAU},
      End_collect
0.00  End_block
(Peak_2)
0.00  Base SameAsMain
0.00  OutletValve F4
0.00  End_block
(End_collect)
0.00  Base SameAsMain
0.00  OutletValve F1
0.00  End_block
```

Here, the second Watch instruction will annul the first since a signal can only be watched for one condition at a time. In the correct construction above, the watch to end collection is not set until Peak_2 is called.

5.9 The Column list

It is possible to select a column in combination with a template when creating a new method (Section 5.1). Certain column-specific parameters are automatically copied into the method. Available columns are found under parameter **Column** in the **New Method** box and new columns can be added to this list and then be selected when a method is created. The Column list is not linked to a particular method, although the columns are edited within the Method editor. Columns are either globally available, i.e. available to all users, or personal, i.e. not available to other users. It is best not to edit the globally available columns unless you save the changes under a new column name, since other users may not appreciate the changes. It is recommended that restricted access be given to editing global columns (Section 14.2).

5.9.1 Adding a new column

To add a new column to the Column list, do the following:

1. Select **File:Column List** to open the **Column List** dialogue. Selection of a column in the list will display its parameters in the field to the right of the box.

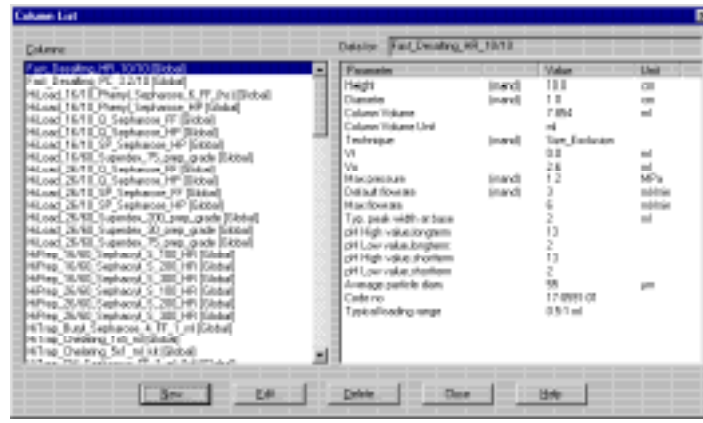


Figure 5-52. The Column List dialogue.

2. Click on **New** to display the **Edit column** dialogue.



Figure 5-53. The Edit column dialogue.

3. In the text box, enter a value for the parameter shown to the left of the text box and click on **Replace**. The value is automatically entered into the **Parameter** list below. New parameters are automatically presented for you to enter values. Repeat this process for those parameters that you have information. Remember to click on **Replace** after each new value has been entered.

Some of the parameters are mandatory (labelled mand) and you cannot save the column until these have been filled in.

The column parameters are used in UNICORN in the following ways:

Parameter	*Unit*	*Mandatory*	*Comments*
Height	cm	yes	Calculation of N/m. Calculation of column volume
Diameter	cm	yes	Calculation of volumetric flow, from a set linear flow. Calculation of column volume
Column volume	ml	yes	Used in method with CV as base. Automatically calculated from **Height** and **Diameter**. Not possible for user to set directly
Column volume unit	ml or litre	no	The column volume is calculated in the set unit. The set unit is also used for Vt, Vo and flow rates
Technique		yes	Decides for which technique the column should be available when **New:Method** is selected
V_t	ml or litre	no	Total liquid volume, used to calculate the capacity factor after an integration

V_o	ml or litre	no	Void volume, used to calculate K_{av} after integration
Max pressure	MPa	yes	Used for setting pressure limit automatically in method. Warning when method is saved and started if the pressure limit is set to higher value
Default flowrate	ml/min or litre/min	yes	Used to set the flow rate automatically in a method. If the method uses linear flow, the parameter value is automatically calculated
Max flowrate	ml/min	no	Used to give a warning when saving or starting a method if a higher flow rate is chosen
Typical peak width at base	ml	no	Used to set averaging time for UV detector. Used to set peak fractionation parameters
pH High value, longterm		no	Used to give a warning when saving or starting the method if the BufferPrep_pH is higher than the set value
pH Low value, longterm		no	Used to give a warning when saving or starting the method if the BufferPrep_pH is lower than the set value

pH High value, shortterm		no	Used to give a warning when saving or starting the method if the BufferPrep_pH is higher than the set value
pH Low value, shortterm		no	Used to give a warning when saving or starting the method if the BufferPrep_pH is lower than the set value
Average particle diameter	µm	no	Information only
Code No		no	Information only
Typical loading range	mg	no	Information only

Note 1: The values for the parameters Max pressure, Default flowrate and Typical peak width at base (used to set average time and peak fractionation parameter MinWidth) are only copied into the method if the corresponding instructions are available as variables.

Note 2: For BioPilot and BioProcess, only the column volume is copied into the method.

4. Click on **Save as** and enter the name of your column. You can choose to save the column globally, i.e. available to all users, by checking the **Save as global** box. If the column is instead for your personal use only, deselect (uncheck) the **Save as global** box. A personal and global column cannot be given the same name. Click on **Save as** again to add your column to the column list.

Note: Saving column globally requires **Edit global lists** authorisation in the user profile (Section 14.2).

5.9.2 Editing column parameters

Column parameters may be edited by doing the following:

1. Select **File:Column List**. Select a column and then **Edit** to display the **Edit column** dialogue.

2. Select a parameter and make the appropriate change in the field above the list. Click on **Replace** to effect the modification. Repeat this process for any other parameters that you wish to edit.

3. To save the changes in place of the original parameters, click on Save. The original column name will be thus retained.

   To save the changed parameters as a new column, click on **Save as** in the **Edit column** dialogue. In the **Save as** dialogue you can change the name of the column. Select (check) the **Save as global box** if you want the column to be globally available. If the column is instead for your personal use only, deselect (uncheck) the **Save as global** box. Click on the **Save as** button and the new column will be added to the column list.

Note: If parameters for a certain column are changed and the same column was earlier selected and saved in a method, the column in the method will not automatically be updated with the new parameters. When opening the method, a question will appear asking if you want to update the parameters. It is recommended that you answer **Yes**.

5.9.3 Deleting a column

To delete a column from the list, mark the column and click on **Delete**. Be restrictive in deleting global columns since these are available to all users.

5.9.4 Selecting or changing the column in a method

Any column may be selected together with the appropriate template in the **New Method** dialogue (Section 5.1). The instruction can later be changed in the Instruction box.

It is also possible to adapt a method to different columns if the column definition name is defined as a variable in the Base instruction (as it is in the method templates). Changing the column for a method is then done in the Variables page.

5.10 Creating and editing BufferPrep recipes

The BufferPrep function is only available to some ÄKTAdesign systems users. Recipes can be edited or new ones created by selecting **File:BufferPrep recipes** in the Method editor. The list of recipes is not linked to a specific method, although editing is performed in the Method editor. Recipes are either global available, i.e. available to all users, or personal, i.e. not available to other users. It is best not to edit the global available recipes unless you save the changes under a new recipe name, since other users may not appreciate the changes. It is recommended that restricted access be given to editing global recipes (Section 14.2).

Note: The recipe to use in a certain method is selected in the BufferPrep page in the Run setup (Section 5.6.8).

5.10.1 Creating a BufferPrep recipe

To create your own recipe do the following:

1. Open the **BufferPrep recipes** dialogue by selecting **File:BufferPrep Recipes** in the Method editor.

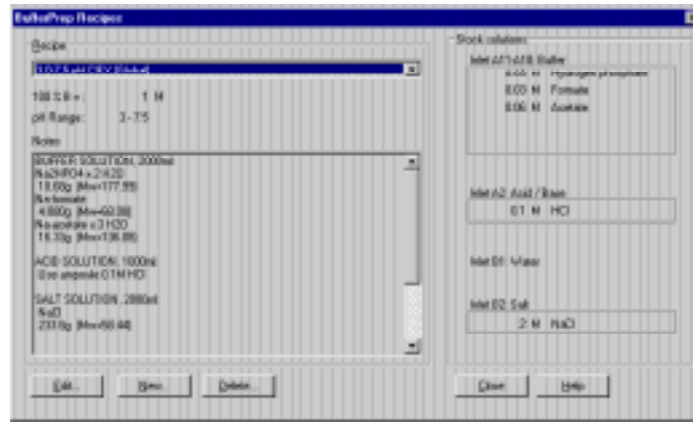


Figure 5-54. BufferPrep recipes dialogue

2. Click on **New** to open the **New Recipe** dialogue and select a buffer from the **Buffer substances** pull-down list. If the required buffer is not listed, see Defining a new buffer substance (below).



Figure 5-55. The New Recipe dialogue.

Insert the intended stock concentration for the selected buffer. Use a buffer concentration of 2-4 times higher than the concentration used in the normal preparation. When BufferPrep is used, the buffer will be diluted between 2-10 times dependent on the amount of acid/base that has to be used to reach the desired pH.

Up to five buffering components can be selected for in the **Buffer** field. To prevent too high ionic strength, the sum of the concentrations for all buffers selected should be between 0.03 M and 0.2 M (typically 0.1 M).

3. Select either **HCl** (acid) or **NaOH** (base) from the **Acid/Base** pull-down list and insert the required stock concentration (typically 0.1 M).

4. Select a salt from the pull-down list. To create new salts; see Defining a new salt (below). Set the maximum outlet concentration of the salt for 100%B (usually 1.0 M).

5. Enter the desired pH range of the buffer.

Note: To set a useful pH range the pKa must be known. Click on the **Buffer substance** button and select the buffer component. The pKa values are shown in the list. Typically, a pH range ±0.5 units around the pKa is useful. For exact ranges check buffer tables.

6. To add notes about the recipe, click on **Notes** and enter the required text in the displayed box. Click on **OK**.

7. When you have created your recipe, click on **Save as**. If you have entered values into your recipe that are not feasible, a warning

message will be displayed that tells you what is wrong. You are given the choice to ignore this warning or to return to the recipe and make the necessary modifications.

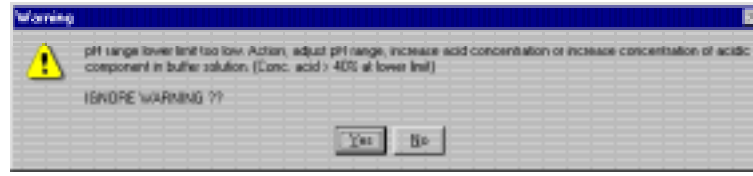

Warning
pH range lower limit too low. Action: adjust pH range, increase acid concentration or increase concentration of acidic component in buffer solution. (Conc. acid > 40% at lower limit)
IGNORE WARNING ??
Yes
No

Figure 5-56. The Warning box is displayed when trying to save a recipe with questionable parameters. This warning suggests that the lower pH range limit is too low and offers possible actions.

Enter a name and save the recipe either as global or personal by checking/unchecking the **Save as global** box. Click on **Save as** to add the recipe to the BufferPrep list.

Defining a new buffer substance

Before defining a new buffer substance ensure that all pKa values for the substance are available. The pKa values entered should be true pKa (i.e. the pKa value at indefinite dilution) as opposed to apparent pKa (i.e. measured at a non-zero concentration). The pKa values should be given at 25°C.

1. In the **New Recipe** dialogue, click on the **Buffer substance** button. Click on **New** button in the **Define buffer substance** box that appears and enter the name of the new component. Click on **OK**.



Figure 5-57. The Define buffer substance dialogue.

2. Select each pKa parameter in turn and enter the appropriate values in the field above the list. Click on **Replace** to enter each value. Up to three values can be entered for each buffering component. When the buffering component has less than three pKa's, the other values should be set to zero.

3. Enter the dpKa/dT values (the change in pKa with respect to change in temperature) for each pKa. Zero means that the pKa does not change with temperature.

4. Enter the number of acidic protons for the buffer substance in the form that is actually weighed in, e.g. for NaH_2PO_4 enter 2, for Na_2HPO_4 enter 1, for Tris enter 0. Click on **Replace**.

5. Enter the charge of the completely de-protonated ion. This will be a negative value for an acid and zero for a base, e.g. for NaH_2PO_4 enter -3, for Tris enter 0. Click on **Replace**.

6. Click on **OK** to add the new buffer substance to the list of available buffers.

Defining a new salt

When defining a new salt ensure that the new salt is inert, i.e. a salt with no buffering properties.

1. In the **New Recipe** dialogue, click on the **Salt** button. Click on **New** button in the **Define salt** box that appears and enter the name of the new component. Click on **OK**.

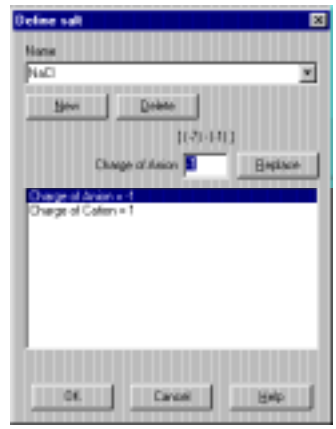


Figure 5-58. The Define salt dialogue.

2. Enter the charge of the anion, e.g. for Cl^- enter -1, for SO_4^{2-} enter -2.

3. Enter the charge of the cation, e.g. for Na^+ enter 1, for Mg^{2+} enter 2.

4. Click on **OK** to add the new salt to the list of available salts.

5.10.2 Editing a BufferPrep recipe

It is possible to edit an existing recipe or a recipe that you have created.

1. Select **File:BufferPrep Recipes** in the Method editor. The **BufferPrep recipes** dialogue will be displayed.

2. Select a recipe from the pull-down list and all information about the recipe will be displayed.

3. Click on the **Edit** button to display the recipe parameters.

   Change the recipe (see Section 5.10.1 for a description of the functions and see below for a description of correction factors) and save using either **Save** or **Save as**. The original recipe will now be updated or a new recipe added to the list respectively.

Note: If parameters for a certain recipe are changed and the same recipe was earlier selected and saved in a method, the recipe in the method will not automatically be updated with new parameters. When opening the method, a question will appear asking if you want to update the parameters in the method recipe. It is recommended that you answer **Yes**. The question will not appear if you only change the correction factors. The correction factors in the method recipe will not then be updated.

Fine tuning with correction factors

In order to obtain high pH accuracy a recipe can be fine tuned around a specific pH by setting correction factors. Run BufferPrep manually at 0% and 100% and change the default correction factors as follows:

1. Select the **Recipe** under group **Other** in **System Control:Manual**. Click on **Execute**. Set the pH in the instruction BufferPrep_pH in group **Pump**. Click on **Execute**. Set the flow rate to be used during the run in instruction **Flow**. Click on **Execute**.

2. Check the pH reading when stable. Allow at least 30 ml of eluent to pass through before expecting a steady pH reading.

3. Change to 100% B by setting the Gradient instruction in **Manual:Pump** to 100% for **Target** and 0 for **Length**. Click on **Execute**.

4. Check the pH reading when stable at 100% B.

5. If the readings are acceptable at both 0% and 100%, the correction factors do not need to be changed.

6. If the readings are not acceptable, click on **Correction factors** in the **Edit Recipe** dialogue (**File:BufferPrep recipes:Edit** in the Method Editor).

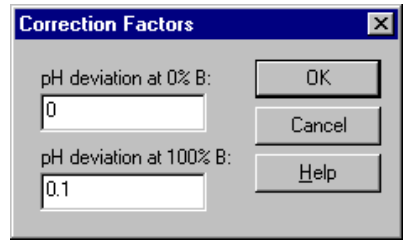


Figure 5-59. The Correction factors dialogue.

7. Enter the deviation at 0% and 100%. (Example: if the pH is set to 7.0 and the actual pH is 7.1 enter 0.1, if the actual pH is 6.9 enter -0.1).

Note: If correction factors already exist, the measured pH deviation should be added to the old factors.

8. Save the recipe.

6 Performing a run

This chapter describes how to perform and monitor runs from the System control module. Calibration of system monitors is also described.

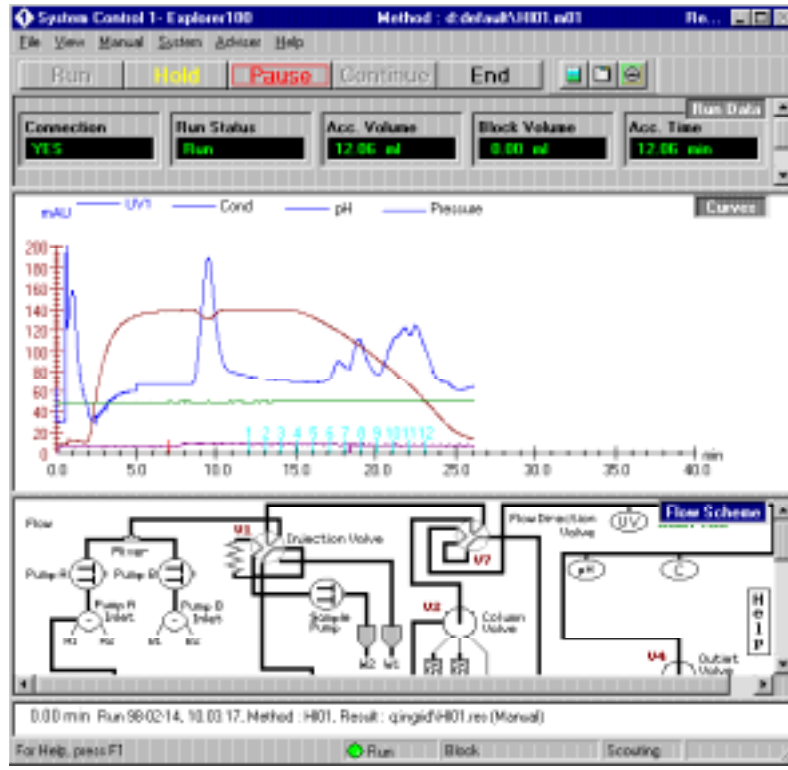


Figure 6-1. The System control workspace with run data, curves, flow scheme and logbook displayed.

6.1 Starting a method

You can only start a method if the system is connected and no method is currently running. You must have **Run methods** authorisation to start a method.

Before starting a method, make sure that:

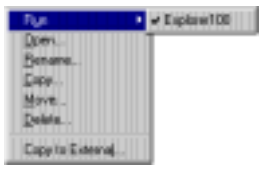
- The correct system is connected in control mode (see Section 6.5).

  The name of the connected system is shown in the title bar of the System control workspace.
  In the status bar information is displayed about the following:
  traffic light with status text,
  current running block,
  Scouting/Record On text,
  MethodQueue text,
  connection status text.

  If the correct system is not connected, you can connect up to the system if it is free by selecting **System:Connect**.

- The system monitors are correctly calibrated (see Section 6.6). Some monitors (notably pH monitors) should be calibrated every day for reliable results. Calibrating system monitors requires **Calibrate/Tune** authorisation in the user profile (see Section 14.2).

6.1.1 Starting from the Main menu



You start a method from the Main menu by selecting the method in the **Methods** window and selecting **File:Run**. Alternatively, you can click on a method file with the right mouse button and select **Run** followed by selecting one of the displayed available systems that have the same strategy as the system for which the method was created. By default, the system for which the currently selected method was created, is selected (checked). You can choose the default system or one of the other systems in this cascade list to run the method.

Note: Do not double-click on the method as this will open the Method editor with the method loaded.

6.1.2 Starting from System control

You can also start a method from the System control module. Click on the System control icon in the Windows NT taskbar. If you have more than one system installed, make sure you select the correct System control icon for the correct system.

From System control select **File:Run** and double-click on the method icon in the displayed dialogue.

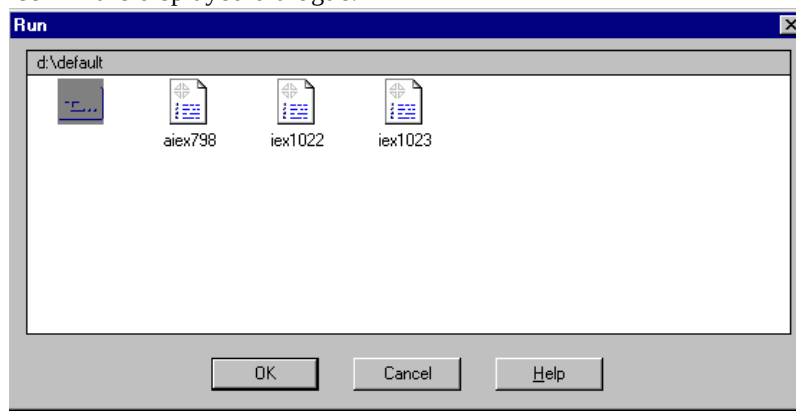


Figure 6-2. Starting a method from the System control menu.

For methods which are used frequently (e.g. column cleaning methods or routine separations), it may be convenient to define the methods as commands in the **File** menu. To do this, choose **File:Menu** and select the required method. The method name will appear as a command in the **File** menu, and choosing the command will start the method.

6.1.3 Starting an Instant Run



You can Start a method template directly by clicking on the **Instant Run** button in the Main menu toolbar or by selecting **File:Instant Run** in System control. The method template will not, however, be saved as a method file, but it is possible to retrieve the method from the generated result file.

6.1.4 Start protocol

If the method is defined with a start protocol this will be displayed before the method actually starts. Work through the start protocol, answering questions as required. As each screen is completed, click on **Next** to move to the next screen or **Back** to return to the previous screen. The last screen has a **Start** button to start the run. At any stage, click on **Cancel** to abort the method start.

The following start protocol items may be displayed (see Section 5.4.4 for more details):

Variables — All the variables defined in the method instructions, organised by block. Values for variables which are not defined as scouting variables can be changed here for the current run.

Scouting	Variables for scouting (see Chapter 7). Values for **Scouting** variables may be changed for the current run and override the corresponding values on the Variables page. It is also possible to include or exclude runs in the scouting scheme with the **Run/Excluded** buttons.
Text Method	Text instructions for the method. These are displayed for information only and cannot be changed at this stage.
Questions	Questions are data entry fields which are filled in by the operator when the run is started. Some questions may be mandatory and some may require authorised confirmation.
Notes	Method notes are shown and start notes can be entered.
Gradient	Displays the gradient for the method.
BufferPrep	Displays the recipe selected in the method. The correction factors can be changed for the current run. It is not possible to change recipe at the start of a run.
Columns	Displays columns selected in the method. Select a column and click on **View** to see the details of the column definition.
Reference Curve	Reference curves which may be displayed in the System control workspace during the run can be selected here. See Section 5.6.6 for a description of reference curve selection.
Evaluation Procedures	Evaluation procedures which will be executed automatically after completion of a run can be selected here. See Section 5.6.5 for a description of procedure definition and selection.
Method Information	Information about the method being run.

System settings	Displays the system settings for the run. If the settings are not suitable, cancel the method start, change the settings with the **System:Settings** command (see Chapter 15) and restart the method.
Calibration	Displays calibration data for system monitors. If the calibration is not acceptable, cancel the method start, re-calibrate the monitor(s) with the **System:Calibrate** command (see Section 6.6) and restart the method.
Result Name	The names of the result file and scouting folder are specified here. This page is displayed if there are any other pages in the start protocol. The names may be changed if this is permitted in the start protocol.

If any questions in the start protocol require authorised confirmation, you will be asked for a username and password when you attempt to leave the screen containing the questions. Only users with **Confirm** authorisation may authorise answers to such questions. Each question that requires an authorisation must have a separate authorisation.

Note: If the start protocol for a method in the queue is cancelled, the MethodQueue is paused. Select **MethodQueue:Dislay Running** in the Main menu and Restart or end the run in the displayed dialogue.

6.2 Monitoring a run

The System control workspace displays the status of the current system. There may be up to four System control modules available on UNICORN desktop which may be connected to different systems. Separate systems may be controlled and displayed independently of each other.



Each System control workspace displays up to four windows for monitoring different aspects of the run. Click on the **View Windows** toolbar button or choose **View:Windows** from the menu to select which windows to display.

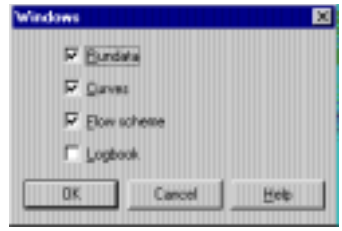


Figure 6-3. Dialogue for choosing windows to display in the System control workspace.

6.2.1 General window techniques

Windows in the System control are always displayed over the full width of the workspace. The boundaries between the displayed windows can be moved by selecting and dragging up or down to change the size of a specific window.

Any window can be maximized to the full view or restored to its original size by selecting the **Maximize** or **Restore** toggle command respectively in the associated right mouse button menu.

To hide a window from view, select **Hide** in the relevant right mouse button menu.

6.2.2 Run data

The run data window displays the current values for selected run parameters. Values are updated at least every 5 seconds (the actual interval is defined in the system strategy).



Figure 6-4. The run data window.

To select the values to be displayed:

1. Select **View:Run Data Properties** or select **Properties** from the right mouse button menu. The **Run Data Properties** dialogue is displayed.

2. Select the **Run Data Layouts** tab.

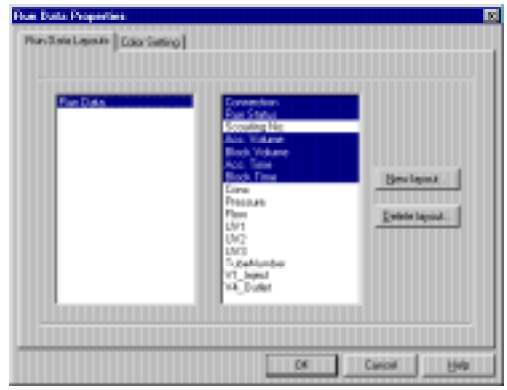


Figure 6-5. The Data Run Properties dialogue, Run Data Layouts tab.

3. Select a layout, either:

an available layout	Select this from the list on the left
edit an available layout	Select this from the list on the left and modify the included readings in the list to the right
create a new layout	Click on the **New Layout** button and enter a name for the layout in the displayed dialogue. Finish by clicking on **OK**. Select the readings that you want to view from the list on the right.

4. To delete a layout, select it and click on **Delete Layout**.

5. Click on **OK** to view the selection in the run data window. The name of the layout selected replaces the default layout name **Run Data**.

6. Toggle between the various layouts that you have created by selecting **Next Layout** in the right mouse button menu.

You can choose run data items to display without using named layouts, simply be selecting or deselecting items in the list. Note, however, that this will change the definition of the currently selected layout.

Run data window style

You can change the colour of the text and background in the run data window.

To change the colour of the text or text background in the displayed readings boxes:

1. Select **Colour Setting:Text** or **Colour Settings:Background** from the right mouse button menu cascade.

2. Select a colour in the dialogue and click on **OK**.

Alternatively:

1. Select **Properties** from the right mouse button menu. The **Run Data Properties** dialogue is displayed.

2. Select the **Colour Setting** tab.

3. Click on the **Text** button or **Background** button and select a new colour. Click on **OK**. The result of the colour change is displayed in the **Colour Settings** tab.

4. Make further adjustments to the colours as appropriate.

5. Click on **OK** to close the dialogue and apply the changes.

6.2.3 Curves

The curves window displays monitor signal values graphically.

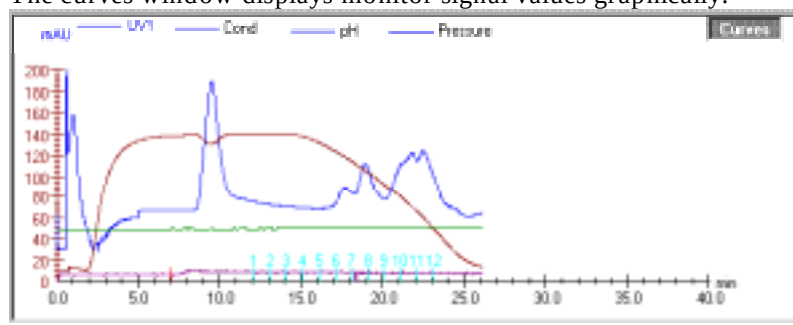


Figure 6-6. The curves window.

To select the curves to be monitored on the screen:

1. Select **View:Curve Properties** or select **Properties** from the right mouse button menu. The **Curve Properties** dialogue is displayed.

2. Select the **Curves** tab.

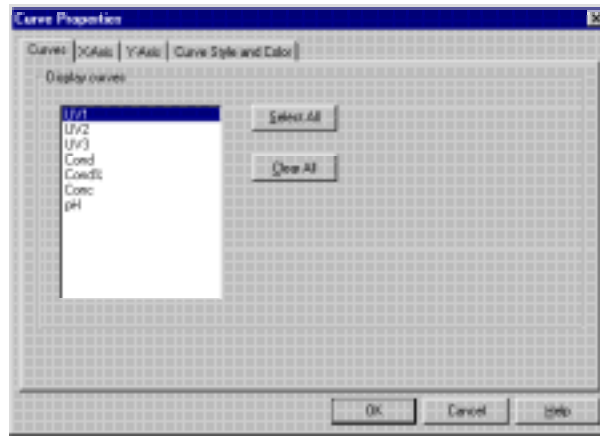


Figure 6-7. The Curve Properties dialogue, Curves tab.

3. Select the curves to be displayed from the list or click on **Select All** if you want to view all of the available curves. To clear the selection, click on **Clear All**. The curves in this list are those for which Store is set to On in the system settings (see Section 15.4) together with any reference curves defined in the method.

Note: Fraction marks and injection marks will always be shown and are not curves in the list.

4. Click on **OK**.

Vertical cursor line

To display a vertical cursor line select **Marker** from the right mouse button menu. Drag the cursor line with the mouse. Where the line bisects the curve, the X-axis and Y-axis values are displayed at the top of the window.

Changing the curve colours and styles

The curves window displays graphs for the selected curves in different colours, with any reference curves included with the method as dashed lines. The curve colours and styles can be changed:

1. Select **View:Curve Properties** or select **Properties** from the right mouse button menu. The **Curve Properties** dialogue is displayed.

2. Select the **Curve Style and Colour** tab.

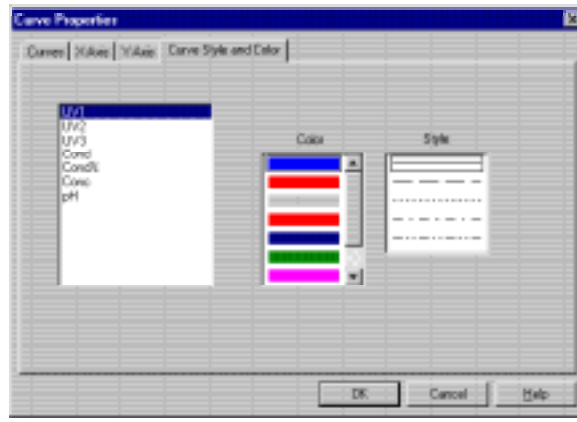


Figure 6-8. The Curve Properties dialogue, Curve Style and Colour tab.

3. Select a curve from the list and then select an appropriate colour and style.

4. Click on **OK**.

Changing the scale of y-axis

The y-axis is automatically scaled for each of the curves. To fix the scale of individual curves:

1. Select **View:Curve Properties** or select **Properties** from the right mouse button menu. The **Curve Properties** dialogue is displayed.

2. Select the **Y-axis** tab.

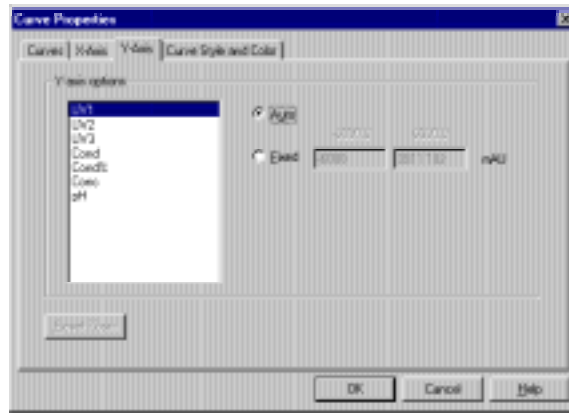


Figure 6-9. The Curve Properties dialogue, Y-Axis tab.

3. Select the appropriate curve and click on the **Fixed** button. Enter a minimum and maximum range in the fields within the specified limits.

4. Repeat steps 2 to 3 for other curves.

5. Click on **OK**.

Values on the y-axis apply to the curve with the same colour as the axis markings. Click on the legend to get the correct Y-axis.

Changing the scale of x-axis

Click on the x-axis to switch the display between time and volume units. (The run is controlled according to the time/volume base defined in the current block, regardless of the base in the curves display).

Alternatively, select the x-units in the **X-Axis** tab of the **Curve Properties** dialogue.

You can also set the viewed portion of the total run.

1. Select **View:Curve Properties** or select **Properties** from the right mouse button menu. The **Curve Properties** dialogue is displayed.

2. Select the **X-Axis** tab.

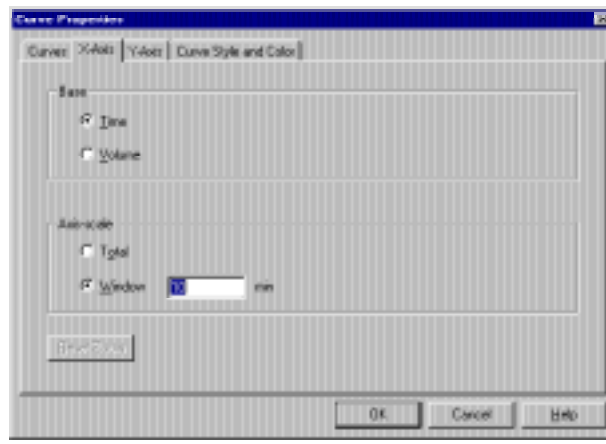


Figure 6-10. The Curve Properties dialogue, X-Axis tab.

3. Select the appropriate base, **Time** or **Volume**.

4. Select the appropriate **Axis scale**, either **Total** or **Window**. The **Total** option will show the curves as far as they have come in the run. The **Window** option allows you to set the portion of the total window to be displayed, either in minutes or ml depending on the selected base.

5. Click on **OK**.

The zoom function

To zoom in on a selected region of the curve window:

1. Press down and hold the left mouse button and drag a rectangle out on the screen to encompass the area to be viewed.

2. Release the mouse button. The display is now zoomed in on the selected area.

3. Repeat the process for further magnification of selected areas.

You can reduce the scale of the zoom in function in two ways, either:

- reverse each zoom in action a step at a time by displaying the right mouse button menu and selecting **Undo Zoom**, or,
- reverse all of the zoom in actions to the default scale setting by displaying the right mouse button menu and selecting **Reset Zoom**

Viewing hatch marks

You can display a grid in the curve window by selecting **Hatch** from the right mouse button menu.

6.2.4 Flow scheme

The flow scheme is a graphical representation of the chromatography system. During a run, the flow scheme displays open flow path(s) in colour and monitor signals with numerical displays. The flow scheme thus shows the current status of the run at a glance.

The flow scheme can be stretched to fit the screen by selecting **Stretch** from the right mouse button menu.

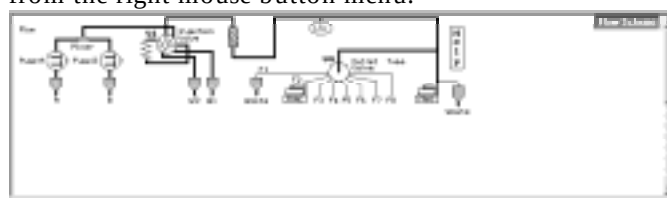


Figure 6-11. The flow scheme for a run.

6.2.5 Logbook

All actions (including method start and end, base instruction, method instructions and manual interventions such as **Pause** or **Hold**) and unexpected conditions such as warnings and alarms are logged for every run, with date, time and current username where appropriate. (The date and time are taken from the system clock in the PC.) The logbook thus provides complete history of any given run. The log is saved in the result file.


Logbook
0.00 min Run 96-02-14, 10:03:17, Method : NOT, Result : q:\ngrid\NOT.res [Manual]
0.00 min Call BASIC_START_CONDITIONS_IEX
0.00 min ColumnPosition Position1Bypass
0.00 min Wavelength 280 nm, 0 nm, 0 nm
0.00 min AveragingTime 0.32 sec
0.00 min Alarm_Pressure Enabled, 5.00 MPa, 0.00 MPa
0.00 min BufferValveA1,A11
0.00 min Gradient 0.00 %B, 0.000 CV
0.00 min BufferPrep_pH 0.000
For Help, press F1
Run
Block
Scouting

Figure 6-12. The logbook panel for a running method.

6.3 Manual control

6.3.1 The toolbar

The toolbar at the top of the System control workspace contains a set of buttons for starting and stopping the run, accessing run notes and locking the system.



Figure 6-13. The toolbar in the System control workspace.

Run — Starts a run when the system is in **End** state and a method is loaded.

Hold — Suspends execution of a method, but continues to pump liquid at the current flow rate and eluent concentration settings. Accumulated time and volume continue to be incremented.
Any method instructions which are set to the time/volume when **Hold** is pressed are executed. Later method instructions are not executed until **Continue** is pressed.

Pause — Behaviour on Pause is strategy dependent. Suspends execution of a method and stops all pumps so that the system comes to a standstill. In BioProcess System, valves are also set to their default position. In BioPilot

System and ÄKTAdesign systems, valves remain in the position they were in before the pause. Accumulated time and volume is not incremented during **Pause**.

Any method instructions which are set to the time/volume when **Pause** is pressed are executed. Later method instructions are not executed until **Continue** is pressed.

Continue — Resumes execution of a paused or held method.

End — Terminates method execution and puts the system into **End** state.

These commands can also be located under the System control **Manual** menu.

The available buttons in System control are dependent on the control status of the connection:

Status	*Available buttons*
End	**Run**
Running	**Hold**, **Pause**, **End**
Manual	**Run**, **Pause**, **End**
Hold	**Pause**, **Continue**, **End**
Method pause	**Hold**, **Continue**, **End**
Manual pause	**Run**, **Continue**, **End**

Other buttons on the toolbar are:



Opens a dialogue for choosing which window panels to display. Clicking on this button is equivalent to choosing the menu command **View:Window**.



Opens the Run setup pages. Run notes may be entered in the Notes page and scouting settings may be changed for scouting run that have not yet started. Other pages are displayed for information only.



The connection mode button has three states which indicate and change the connection mode (see table below).

.

Button	*Connection mode*	*Click to change mode*
	System control workspace disconnected	Click to connect to a system
	Control mode connection	Click to leave system
	View mode connection	Click to establish a control mode connection (if possible)

The status bar also displays a text message indicating the connection status of the window:

Controlled by :<user> The indicated user has a control mode connection to the system. Other users may establish a view mode connection.

Locked by: <user> The indicated user has left the system in a locked state. Users who can supply the required password may unlock the system and establish a connection. Note that the password is case sensitive.

Note: It is possible to unlock with the “lock” password or with the UNICORN logon password. If using the UNICORN logon password, the user must have the **Unlock systems** access rights. The “lock” password is the password entered by the user who locked the system and is case sensitive.

System is available Any user may establish a connection.

System connections are described in more detail in Section 6.5.

6.3.2 Manual instructions

The chromatography system can be controlled with manual instructions issued from the **Manual** menu. To save the results of a manual run, issue the instruction Record_on (in the **Other** instruction group) at the beginning of the run. UNICORN will prompt for a result file name at the beginning of the run.

The **Manual** menu opens a dialogue similar to the text instruction box in the Method editor (see Section 5.4):

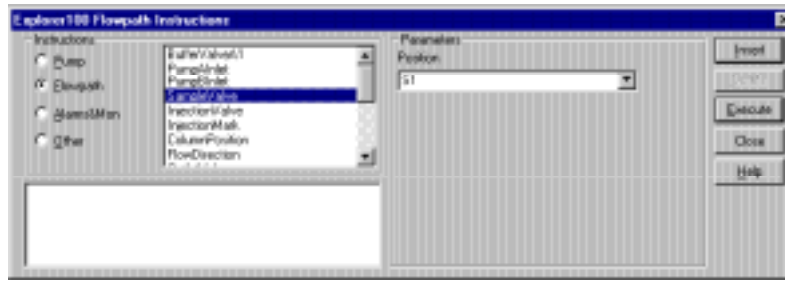


Figure 6-14. The Manual instruction dialogue.

The name of the connected system is displayed in the title bar of System control. Available instructions are determined by the strategy for the connected system.

Manual instructions are entered in the same way as method instructions from the dialogue in the Method editor. The **Insert** button places the current instruction in the list at the bottom left of the dialogue. Clicking on **Execute** executes all instructions in the list at the same time, or executes the currently marked instruction if the list is empty. Note that although all instructions are executed simultaneously, some (for example gradient and fraction instructions) may take some time to complete in the liquid handling module.

The **Delete** button deletes selected instruction from the current list. Only one instruction can be deleted at a time.

If you close the dialogue by clicking on the **Close** button without choosing **Execute**, commands in the list will not be executed and will be deleted from the command list. Manual instructions can also be issued while a method is running. A manual setting applies until the next method instruction of the same type is executed (e.g. a manual Flow instruction will set the flow rate until the next Flow instruction in the method is executed). Manual instructions that you issue during a method are recorded in the logbook for the method run.

6.3.3 Alarms and warnings

The system settings (see Section 15.1) determine the acceptable limits of monitor signals during a run. The limits can also be set for the current run using an instruction in the method. Limits set with a method instruction override the limits set in system settings. If these limits are exceeded in a run, a warning (W) or alarm (A) dialogue with a message is displayed on the screen.

- The run continues if a warning is issued.
- An alarm pauses the system.

Warnings and alarms are displayed regardless of the activity currently in progress in UNICORN: you will be notified of an exceeded limit in a running system even if you are developing a method, evaluating data or monitoring a run on a different system. Warnings and alarms are also recorded in the logbook for the run.

In a network installation alarms and warnings are displayed on the controlling station and all stations viewing the system. An alarm can be acknowledged only from the computer connected in control mode: alarms are displayed but cannot be acknowledged on computers connected in view mode.

Note: For this reason, we discourage "passive" operation of a system, i.e. with no controlling connection.

6.4 If communication fails

This section summarises the consequences of system failure during a run. See Section 11.3 for more details.

If network communication fails during a run that has been started from a remote station, the run will continue and the results will be saved in the Failed folder on the local station. A control mode connection can be established on the local station to control the running system.

6.5 Managing system connections

UNICORN installed on a given computer may have up to four System control workspaces (the actual number is determined when the software is installed, see Chapter 13), each of which may be connected to one chromatography system at a time. Connections are managed using the **Connect** and **Disconnect** commands in the **System** menu. A network installation may have more than four systems in total, but each computer in the network can establish a maximum of four connections. Connection management is the same for stand-alone and network installations.

6.5.1 Establishing a connection



To connect a System control module to a chromatography system, open a System control that is not currently in use (identified by the Disconnected icon in the System control tool bar) and choose **System:Connect**. The dialogue lists the systems to which you have access. Select the system to which you want to connect and click on **OK**.

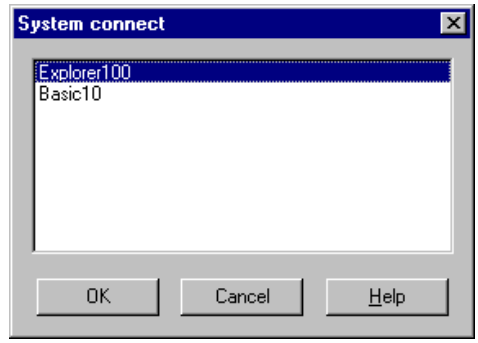


Figure 6-15. System connect dialogue.

To connect to a system from a remote station in a network installation, the local station (i.e. the computer physically connected to the chromatography system) must be logged on to the network. UNICORN does not however need to be running on the local station.

A local station can be used to control the chromatography systems directly connected to the PC without logging on to the network. Files stored on network drives will of course not be accessible. This mode of working places UNICORN into an "error" state and you should not ideally work in this state since global files such as the user settings file (musers30.mpm) etc. are stored on the network. Any changes made to these files while not logged on will apply locally and will be lost the next time you log on to the network to use UNICORN. For runs performed in this stand-alone mode where the result file is directed to a network drive, the results will be saved in the Failed folder on the local station (see Section 11.3).

Several simultaneous connections can be established to one system, but only one may be in control mode, i.e. able to actively control the system. The other connections are in view mode, and can monitor the system activity but cannot issue commands.

6.5.2 Connection modes

The possible connection states of a System control module are indicated by the connection mode button and the status text on the status bar as summarised below:

Button	Connection mode	Text	State/Action
	Not connected	(none)	Click on the connection mode button or choose **System:Connect** to establish a connection.
	Control mode	**Controlled by: <user>**	Click on the connection mode button to leave the system but retain the connection with the System control module. You may leave the system locked or unlocked.
	View mode	**Controlled by: <other user>**	The indicated user has a control mode connection. Clicking on the connection mode button has no effect.
	View mode	**Locked by: <other user>**	The indicated user has left the system in a locked state. Click on the connection mode button to establish a control mode connection (you must supply the locking password, which is case sensitive, or your logon password if you have Unlock locked systems authorisation).
	View mode	**System is available**	A user has left the system in an unlocked state. Click on the connection mode button to establish a control mode connection.

In all modes you can choose **System:Disconnect** to disconnect the system from the control window.

6.5.3 Leaving and locking a system



A running or **End** state system with a control mode connection can be left and locked by clicking on the control mode button or selecting **System:Leave system**. When the system is left, the connection becomes a view mode connection. After leaving and locking scouting or MethodQueue runs, it is not possible to establish a control mode connection from another computer.



Figure 6-16. Leave Control dialogue.

You may leave the system unlocked or locked:

- **Unlocked** leaves the system unlocked. Any other user may establish a control mode connection to the system. Use this option if you do not intend to use the system in the near future.
- **Locked** locks the system with the password specified in the dialogue. A control mode connection can only be established by providing the correct password. Note that this password is independent of the user's logon password. A locked system can also be unlocked with the logon password for a user with **Unlock locked systems** authorisation. This authorisation should be restricted to a small number of users to prevent indiscriminate unlocking of locked systems.

6.5.4 Disconnecting a system

To disconnect a chromatography system from a System control module, choose **System:Disconnect**. If you are disconnecting a control mode connection, you will be asked to leave the system first.

Logging off or quitting UNICORN automatically disconnects all connected systems, displaying the **Leave control** dialogue for each system. Systems which are disconnected in this way will be re-connected automatically when you log on to UNICORN again. (Note however that you may have disconnected from a control mode connection but establish a view mode connection on re-connect, if another user has taken control of the system in the meantime).

Note: You can disconnect a system during a run and the run will continue. It is not recommended to do this without locking the system, since this can leave a run on the system with no responsible user. You cannot however disconnect from scouting or MethodQueue runs.

6.5.5 Network considerations

In a network installation, a chromatography system can be controlled from any computer in the network provided that the user has sufficient access rights in UNICORN. UNICORN software has to be installed but not necessarily running on the computer to which the system is physically connected. The computer has to be logged on to the network.

- A system which is controlled by another user through a control mode connection can be viewed through a view mode connection by any user with sufficient access on any number of computers in the network. This allows runs to be monitored from multiple display stations (although only the active connection can control the system).
- A system that is locked by a user can be unlocked on any computer in the network (not possible during scouting or MethodQueue runs) by any user with sufficient access rights (see above).
- A UNICORN user may log on to UNICORN on up to eight different computers in the network. Each successive multiple instance of the user automatically establishes the same System control connections as the first instance when the logon is performed. Multiple instances are however treated by UNICORN as separate users (although they are not distinguished in the System control workspace display) and only one of the instances may maintain a control mode connection to a system. Multiple instances may also disconnect and connect their System control modules independently of each other once the logon is performed. See Section 2.6.2.

6.6 Calibrating monitors

Certain system monitors (notably pH monitors) need to be calibrated regularly for correct results. According to the routines established in the laboratory or process department, monitors may be calibrated at pre-set intervals by the system technician, or calibrated as required by the user before each run. This section describes the calibration procedure.

For ÄKTAdesign systems, the sample pump, pressure offset and pH must be calibrated regularly. You can calibrate pH, conductivity cell constant and temperature in either UNICORN or the monitor.

For BioPilot and BioProcess systems, the pressure and pH monitors must be calibrated.

Monitors can only be calibrated by users with **Calibrate/Tune** access. Calibration cannot be performed while a method is running.

All monitors can be calibrated from UNICORN to convert monitor signals to appropriate units for display. For monitors supplied by manufacturers other than Amersham Biosciences, and for some Amersham Biosciences monitors, calibration in UNICORN should be performed each time the monitor itself is calibrated.

For most monitors supplied by Amersham Biosciences, calibration from UNICORN also performs a true calibration of the monitor, adjusting the signal-response level in the monitor. For these monitors, calibration on the monitor itself (without using UNICORN interface) is recognised by UNICORN and the conversion factor is adjusted accordingly. The conductivity monitor for BioPilot can only be calibrated from the monitor itself.

Calibration may use one-point, two-point or continuous measurement, depending on the type of monitor and system:

One-point calibrations are based on measurements taken at one reference point. The monitor will be calibrated based on an assumed linear response between the reference point and zero.

Two-point calibrations are based on measurements at two reference points. The monitor will be calibrated assuming a linear response between the two reference values. It is important that monitors are calibrated in the measuring range for which they will be used, particularly when the response is not linear over the whole operating range of the monitor (Figure 6-17).

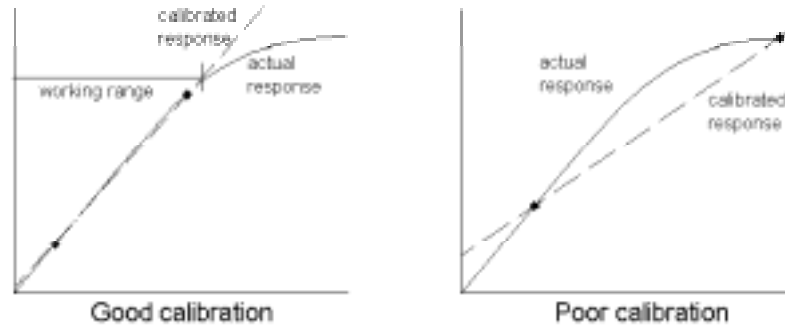


Figure 6-17. For a monitor with a non-linear response, make sure the reference points are within a linear working range.

Continuous calibration is based on a value accumulated during a given time. This kind of calibration applies to the sample pump in ÄKTAdesign systems and on pumps in BioProcess System.

6.6.1 Calibrating monitors for ÄKTAdesign systems

To calibrate the monitors for ÄKTAdesign systems, do the following:

1. Select **System:Calibrate** to display the **Calibration** dialogue.
2. Select each of the monitors in turn and perform the procedures described below.
3. Click on **Close** once the calibrations have been made.

Pressure

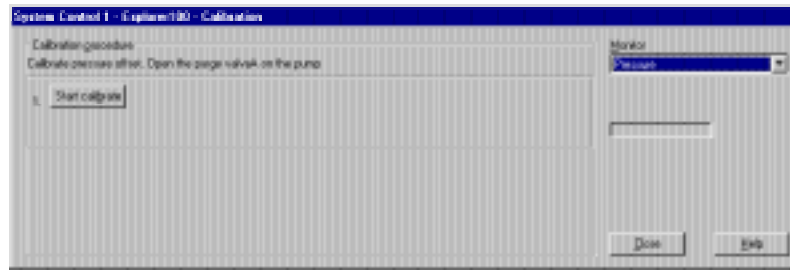


Figure 6-18. Dialogue for setting the pressure reading to zero.

By selecting **Pressure** you can adjust the pressure reading to zero when the actual pressure is zero.

1. Set the flow rate on the pump to zero and open the purge valve for pump A. The actual pressure will then be zero.

2. Click on **Start Calibrate** to set the pressure reading to zero.

Temperature

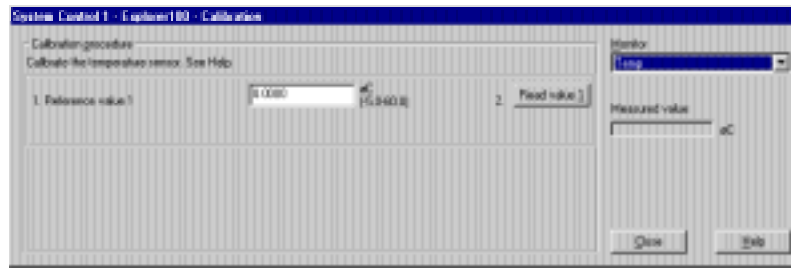


Figure 6-19. Dialogue for temperature calibration in the conductivity cell.

By selecting **Temp**, you are able to calibrate the temperature sensor based on a reference temperature (the temperature sensor is placed into the conductivity cell). For further information, see Section B.2.6 in the Monitor pH/C-900 User Manual. Enter the temperature measured with an external thermometer and click on first **Read Value 1** and then **Exit**.

pH



Figure 6-20. Dialogue for pH calibration.

Some ÄKTAdesign systems has a pH monitor to allow on-line pH measurements. Calibration can be performed with the electrode either within the flow cell or outside.

- If calibrating with the pH electrode sitting in the flow cell:

1. Attach the first pH reference solution, e.g. pH 7.0, to Inlet A11 on the system and the second reference solution, e.g. pH 4.0, to Inlet A12.

2. In System control, select **Manual:Pump**, select the instruction **PumpWash** and then choose **Inlet A11**. Click on **Execute**. The pump and inlet tubings will now be filled with the first reference solution.

3. When the **PumpWash** is finished, click on the **Flowpath** radio button. Mark instruction **ColumnPosition** and ensure that **Position1Bypass** is selected.

4. Click on the **Pump** radio button and select the instruction **Flow**. Insert the flow rate that you will use later during your run and click on **Execute**.

5. Select **System:Calibrate** and select monitor pH.

6. The **Measured value** field shows the actual reading according to the previous calibration. This value may be incorrect and does not affect the current calibration. Allow at least 35 ml of reference buffer to pass through the cell, during which time the reading should stabilise.

7. When the pH is stable, enter the known pH of the reference solution in the field for **Reference value 1**. Click on the **Read Value 1** button.

8. Switch to the second reference solution by selecting **Manual:Pump** and the instruction **PumpWash**. Select **Inlet A12** and click on **Execute**. When the **PumpWash** is finished, repeat steps 6-7 for **Reference value 2**.

9. After the calibration, values are automatically entered into the **Calibrated electrode slope** and **Asymmetry potential at pH 7** fields. A new electrode will have a slope between 95-102% and an asymmetry potential from -30 to +30 mV. Should the values fall outside these ranges, clean the electrode. If the slope falls below 80% and the asymmetry potential outside ±60 mV and cannot be improved by cleaning the electrode, then you must replace the electrode. For further information, read the Monitor pH/C-900 User Manual.

- Calibrating with the electrode outside the flow cell:

See Section 6.6.2 Calibrating monitors for BioPilot, for a step-by-step description. See also the User Manual for Monitor pH/C-900 Section 3.6 for details about calibrating off-line.

Remember to not remove the electrode from Solution 1 until the **Read Value 2** button has become available (the text on the button turns from grey to black).

Calibrating the conductivity cell constant

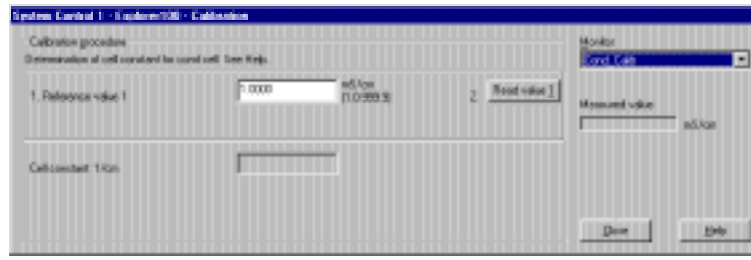


Figure 6-21. Dialogue for calibration of the conductivity cell constant.

By selecting **Cond_Calib**, you can calibrate the conductivity cell constant. Normally it is not necessary to adjust the cell constant as the flow cell is pre-calibrated on delivery. Adjustment is only necessary when the monitor is to be used to determine specific conductivity with high accuracy. See the User Manual for Monitor pH/C-900, Section B.2.2 about calibrating the conductivity cell, for full details. Enter the known conductivity for the calibration solution at the present temperature and click on **Read value 1**. The value for the cell constant will be calculated and then be displayed.

New conductivity cell constant

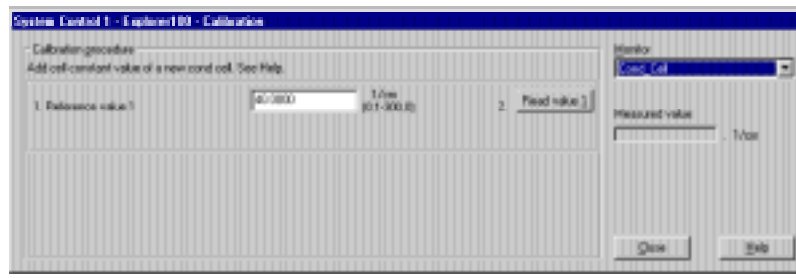


Figure 6-22. Dialogue for entering the calibration constant of a new conductivity cell.

By selecting **Cond_Cell**, you are able to insert the cell constant for a new conductivity cell. Information about the cell constant is supplied on the packaging of the new cell. Insert this value in the **Reference value 1** and click on **Read Value 1**.

6.6.2 Calibrating monitors for BioPilot

Calibration of the pH and pressure monitors for the BioPilot system is achieved as follows:

pH

1. Open the **Calibration** dialogue and select the monitor to calibrate (e.g. **pH1**).

2. Place the electrode in the first reference solution, e.g. pH 7.00, and enter the reference value in the field for **Reference value 1**.

   The **Measured value** field shows the actual reading according to the previous calibration. This value may be incorrect and does not affect the current calibration.

3. When the reading is stable, click on the **Read value 1** button.

Note: When calibrating a pH meter, do not remove the electrode from solution 1 until the **Read value 2** button has become available (the text on the button turns from grey to black).

4. Repeat steps 2-3 for the second reference solution (e.g. pH 4.00) to set **Reference value 2**.

Pressure

1. Open the **Calibration** dialogue and select the monitor **PressureAB**.

2. Select **Manual:Pumps** from the System control menu and start **PumpA** with a low flow rate to give a low back pressure.

3. Read the pressure on the pump's pressure monitor and enter this value in the field for **Reference value 1**. Click on **Read value 1**.

4. Change the flow to a high flow rate or run the buffer through a column to create a higher back pressure. Repeat step 3 to set **Reference value 2**.

5. Press **Save**.

For pressure sensor calibration, the calibration constant and offset values may be entered instead of performing the calibration procedure.

Click on **Save** to save the calibration before closing the **Calibration** dialogue or choosing another monitor.

6.6.3 Calibrating monitors for the BioProcess System

Continuous calibration for pump flow rate is done in the following way:

1. Open the **Calibration** dialogue and select **Flow**.

2. Using manual instructions (see Section 6.3), open a flow path to outlet **Waste** and start the pump at a suitable flow rate, in the range which will be used.

3. Place a collecting vessel under outlet **P1**.

4. Click on **Start collect**. This switches the outlet from **Waste** to outlet **P1**.

5. Collect a suitable volume. It is important that the collected volume is large enough to eliminate the effects of any unevenness in flow rate.

6. Click on **Stop collect**. This switches the outlet from **P1** back to **Waste**.

7. Measure the collected volume and enter the result in the **Collected volume** field. The flow rate is calibrated from this value and the time of collection, measured internally by UNICORN. Click on **Save** when the required monitors have been calibrated.

6.7 Feedback tuning

Feedback tuning (menu command **System:Tune**) tunes feedback control of pumps for flow rate and gradient formation. Gradient control is normally based on conductivity measurements. This option is only available for BioProcess System installations.

The feedback control model is described in Appendix E. Feedback is tuned by entering values for P, I and D at specified setpoints and adjusting these values until the feedback behaviour is satisfactory. A graphical presentation of input, output and setpoint values assists the tuning procedure.

7 Scouting

Scouting is a facility for automatically repeating a run with systematic variation of one or more parameters. Some typical situations where scouting is useful are:

- screening for the best column
- finding the optimal pH
- testing column capacity (sample volume)
- finding the optimal flow rate for binding and elution
- optimising gradient length and slope
- optimising step gradients

This chapter describes how to set up and use scouting schemes in UNICORN.

7.1 Setting up scouting

Scouting schemes are defined as part of the Run setup in the Method editor (see Section 5.6). Any parameters (including breakpoints) can be scouted provided that they can be defined as variables in the method. To set up a scouting scheme:

1. Create a method by selecting a method template. If a method is created without using the method templates, define appropriate variables in the method.

2. Open the Scouting page in Run setup.

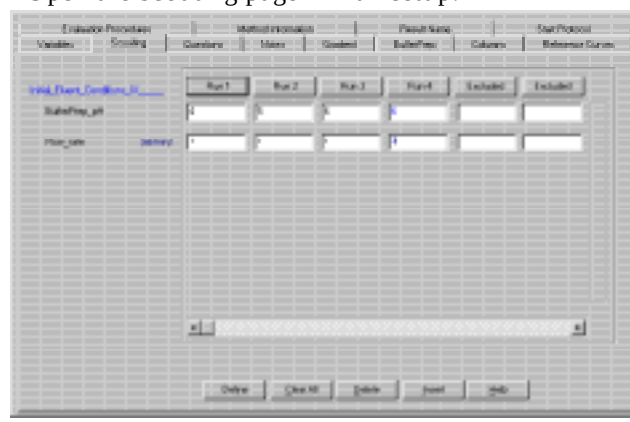


Figure 7-1. The scouting page in Run setup showing a scheme for testing flow rate. The scheme also allows sample volume to be scouted.

3. Click on **Define** and choose the variables you wish to scout from the list.

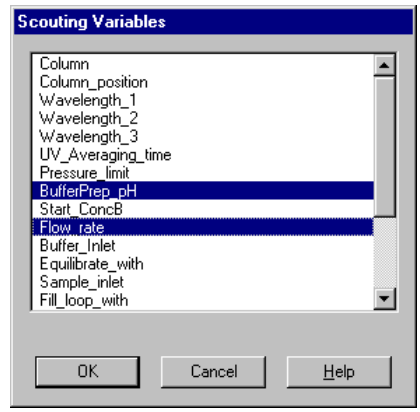


Figure 7-2. The dialogue for selecting scouting variables.

By default, no variables in the method are selected. Select only the variables you wish to scout.

4. Click on any cell in the first empty run column to insert default values for the variables.

5. Make any changes you require in the variable values. Use the scroll bar at the right-hand side of the page if the variables do not fit on to one screen. For variables with text values (e.g. column position), double-click in the variable field and select a value from the list that appears.

6. Click on the next run column to copy the values from the previous run, and change variable values as required.

7. Repeat this procedure until you have defined all the runs you require. You can define up to 99 runs for scouting. Use the scroll bar at the bottom of the page to define more than 6 runs.

   All variables in a run must be assigned values. If you attempt to delete values in a run, UNICORN will insert the lowest acceptable value for the variable.

   You can delete and insert run columns using the **Delete** and **Insert** buttons respectively.

8. If you want to exclude runs from the default scouting scheme, click on the **Run** button at the top of the variable column to toggle between **Run** and **Excluded**.

9. Open the Start Protocol page in Run setup. Check the **Scouting** box if you want the scouting page to be displayed at the start of the run (recommended). This allows the operator to adjust the **Run/Excluded** status for individual runs and the values for scouting variables before the method starts. If this box is not checked, running the method will perform scouting as defined in the method.

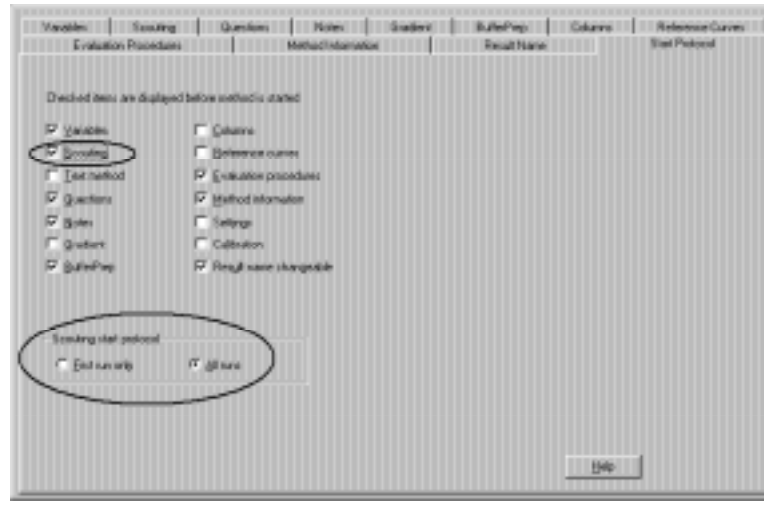


Figure 7-3. Settings on the Start Protocol page in Run setup determine whether the Scouting page will be displayed at the beginning of scouting runs.

If the **Variables** box is also checked, the start protocol will display the Variables page as well as the Scouting page. Values for variables defined for scouting are, however, grey in the Variables page and cannot be changed there.

Under **Scouting start protocol**, check **First run only** if you want the start protocol to be displayed only before the first run. If this box is checked, the settings entered in the start protocol for the first run will apply throughout the run, and the scouting series will be performed automatically without user intervention. Check **All runs** if you want the start protocol to be displayed before each run in the scheme, giving the operator an opportunity to change variable values or e.g. fill the sample loop before each run. The operator must then press Start before each run in the scouting scheme.

10. Open the Result name page in Run setup and enter a name for the scouting folder. Change the path for the result file if required. The scouting results will be saved in a folder on the result file path, named according to the specified scouting folder name with a 2-digit serial number.

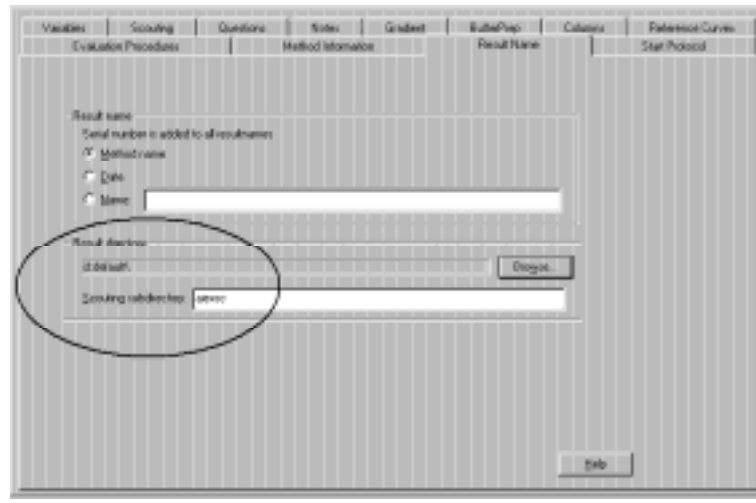


Figure 7-4. Settings under Result folder on the Result page in Run setup determine the name of the folder where scouting results will be stored.

11. Save the method.

7.1.1 Scouting variables

The variables that appear in the scouting scheme are usually a subset of those on the Variables page of Run setup. However, while the default values in the method can be set either on the Variables page or in the text instructions (see Section 5.5), values in the scouting scheme can only be set on the Scouting page. Changing variable values in the scouting scheme does not change the values on the Variables page or in the text instructions. Values for variables selected for scouting are grey on the Variables page and cannot be changed there.

7.1.2 Scheme set-up

Each run column in the scouting scheme represents one potential run through the method. The runs which will actually be performed are defined by the status of the **Run/Excluded** button at the top of each column.

7.1.3 Start protocol settings

If the **Scouting** box in the start protocol is not checked, the scouting scheme will run automatically as defined in the method. (We recommend that the box is always checked for a scouting method. There is otherwise no indication for the operator at the start of the run that the method actually performs several runs).

7.2 Running scouting

To perform a scouting run:

1. Start the method (see Section 6.1). The start protocol will display the scouting scheme as defined in the method (assuming that the Scouting box is checked in the start protocol definition, see above).

2. Check through the settings for the scouting scheme, and if required:

   - change variable values for the runs
   - click on the **Run/Excluded** button at the top of a variable column to toggle the run status between **Run** and **Excluded**.

   If the start protocol is displayed at the beginning of each run, you can adjust the settings for the current and subsequent runs at the beginning of every run. If the start protocol is not displayed at the beginning of each run, the sequence of runs will be performed automatically according to the settings entered in the start protocol for the first run.

3. Work through the rest of the start protocol, then press **Start**.

The results of a scouting run are saved in a special scouting folder as defined in the Results page of the start protocol. Within the folder, each run is saved in a separate result file named according to the usual naming rules (see Section 5.6.9).

Note: If the start protocol is displayed for each run in a scouting scheme, you may be able to change the result file name during scheme execution.

7.2.1 Changing scouting settings during a run



At any time during a run, you can click on the **Run setup** button in System control and change the scouting settings in the Run setup Scouting page for runs which have not yet been started. Settings for the run which is currently in progress cannot be changed.

You can use this feature to adjust settings for scouting even if the start protocol is not displayed at the beginning of each run.

7.3 Scouting results

7.3.1 Viewing results

Each result file in the scouting result folder represents the results of one scouting run, and can be viewed in the same way as result files from ordinary runs (see Chapter 9). The Scouting page in the documentation saved in each scouting result file shows the whole scouting scheme, with the values for the current run displayed in a cell with a yellow background.

Note: Any changes made to the variable values during a run will only be recorded in the result files for runs after the changes were made.

7.3.2 Comparing runs

To compare chromatograms in results from different runs in a scouting series:

1. Open a result file from the scouting series.

2. Select **File:Open to compare** and choose the chromatograms you want to compare (see Section 9.4 for full details). Click on **OK**.

7.3.3 Printing results

Scouting results can be evaluated and reports can be generated in the same way as for other result files (see Chapters 9 and 10). The following settings in **File:Report** in the Evaluation module are important for scouting:

Documentation:Scouting includes the complete scouting scheme, but does not indicate which run generated the particular result file.

Documentation:Variables prints the variable values used during the particular run.

Edit:Chromatogram Layout Box, **Header** tab **Scouting Variables** option prints the scouting variable values for the particular run as part of the diagram header.

For reports generated from scouting runs, always make sure that **Scouting Variables** is selected in **Edit:Chromatogram Layout Box**, **Header** tab so that the scouting variable values for the particular run are included.

7.4 Scouting examples

Below are some typical examples of scouting, illustrating the way scouting schemes can be set up for different purposes.

7.4.1 Scouting sample volume

Sample volume scouting is applicable to both adsorption and gel filtration techniques. Superloop can be filled with sample and the sample volume varied with the Empty_loop_with variable.

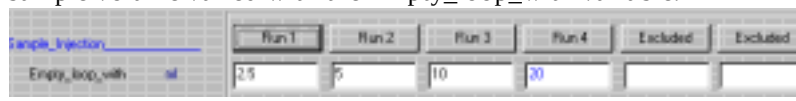


Figure 7-5. Scouting settings for sample injection.

7.4.2 Screening different columns

Scouting can be used to screen different column types for an application.

For screening different columns, a suitable scheme may include the variables:

- Column
- Column_position (switches the ColumnValves to the chosen column)
- Flow rate

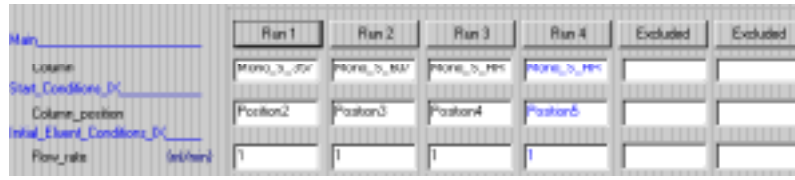


Figure 7-6. Screening different columns, with facilities for changing the column, column position and flow rate for each column.

7.4.3 Scouting continuous gradients

Scouting continuous gradients involves finding the best final concentration and gradient length for elution of the product from a given column. These two parameters are set as variables.

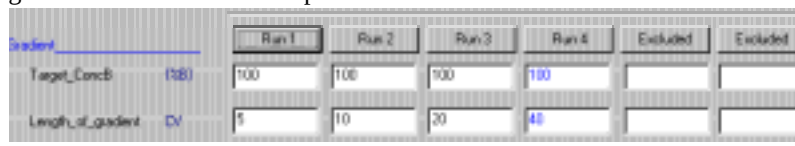


Figure 7-7. Scouting for optimal gradient slope and length.

7.4.4 pH scouting

With BufferPrep it is possible to vary the pH automatically in the different runs.

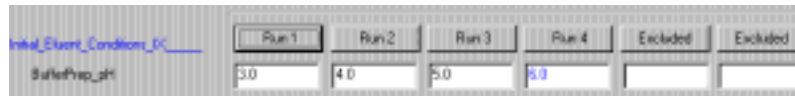


Figure 7-8. Scouting for optimal pH.

8 MethodQueues

MethodQueues provide a means for linking several methods together, on the same or different systems. For example, if a system wash procedure is programmed in a separate method, it can be linked in a MethodQueue to a series of different process methods, ensuring that the same wash procedure is used before every process. Alternatively, the product of a separation on one system might form the starting material for a separation on the next, allowing fully automated multi-step processing.

Specific user authorisation is required to edit and run MethodQueues, separate from that required for editing and running methods.

8.1 Setting up a MethodQueue

8.1.1 Defining a MethodQueue

To create a new MethodQueue:

1. In Main menu select **File:New:MethodQueue** or **MethodQueue:New**. The **MethodQueue Editor** dialogue is displayed.



Figure 8-1. MethodQueue Editor dialogue

2. To add a method to the MethodQueue list, select the **End of MethodQueue** instruction in the list.

3. Click on the appropriate **System #** button. The **Load MethodQueue** dialogue is displayed.

Note: The number of available **System #** buttons is dependent on the number of systems that were selected during installation of UNICORN 3.00.

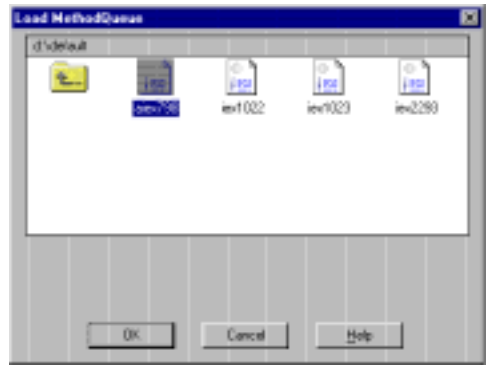


Figure 8-2. Load MethodQueue dialogue.

4. Use the dialogue to locate and select the required method. Click on **OK** or double click on the method item.

   The system type for the selected method, e.g. **Basic 10**, is assigned to the selected **System #** button and the **System #** name on the button is replaced with the name of the system.

   A line is also inserted into the MethodQueue list before the previously selected **End of MethodQueue** instruction. The new line contains the name of the selected method.

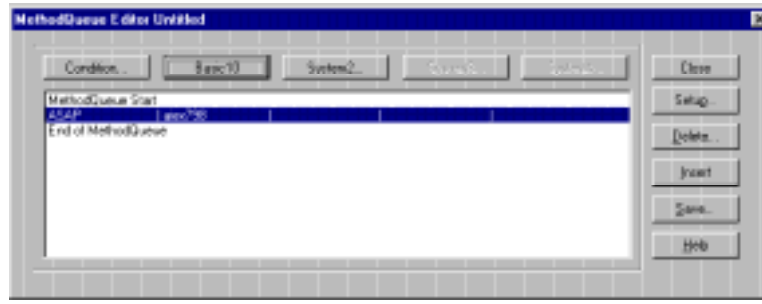


Figure 8-3. MethodQueue Editing dialogue with a method selected for an Basic 10 system.

5. To add more method steps click on the **Insert** button and repeat steps 3-4. Note that you must use a new **System #** button to add methods written for a different system type.

   For example, if you have designated **System 1** to methods for the **Basic 10** system, then only methods written for Basic 10 can be added with this button. If you want to add to the list methods written for, for example, Explorer 100 then you must use another

free **System #** button such as **System 2**. In this case, **System 2** will be assigned and renamed **Explorer 100** and only methods written for this system can be added using this button.

6. By default each method step will start as soon as possible (**ASAP**) after the completion of the previous method step. To set the time interval for starting a selected step click on the **Condition** button. In the **Condition** dialogue set the time when the step is to start. Click on **OK**.

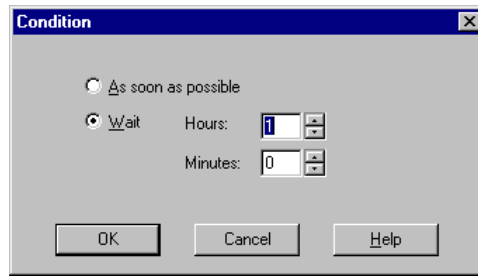


Figure 8-4. Condition dialogue.

The timing of MethodQueue steps performed on different systems can also be controlled by the Ready instruction (see Section 8.3.1) in the method.

7. Click on **Setup** to define the starting time for the MethodQueue. **Immediate start** sets the MethodQueue to start as soon as you request **Run** from the Main menu. **Start time** sets the MethodQueue to start at a pre-set time of day up to one week after **Run** is requested. If no day is specified, the MethodQueue will start as soon as the pre-set time is reached (i.e. within the next 24 hours).

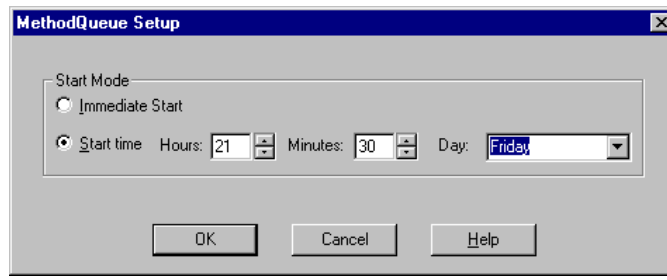


Figure 8-5. MethodQueue Setup dialogue.

Note: The MethodQueue setup time defines the starting time for the MethodQueue as a whole. Do not confuse this with **Condition**, which defines the relative starting time for a step within a MethodQueue.

8. Click on **Save** to save the MethodQueue. Enter a MethodQueue name in the dialogue.

Note: **Edit MethodQueue** authorisation is required to define a new MethodQueue.

8.1.2 MethodQueue folders and icons

MethodQueues are saved in a separate MethodQueue folder within the folder that you specified during the save. The MethodQueue folder is represented by a special icon in the **Methods** window of the main UNICORN menu.



MethodQueue folder.

Double-click on the MethodQueue folder icon to open it. A MethodQueue folder contains the MethodQueue definition and copies of all methods included in the MethodQueue.



MethodQueue definition (in MethodQueue folder).

It is important to realise that the MethodQueue works with copies of the original method files. If changes are made in the original method, these will *not* affect the method in the MethodQueue. To implement changes in a MethodQueue method, edit the method definition in the MethodQueue folder. Alternatively, edit the original method, then use the MethodQueue editor to update the MethodQueue definition, replacing the old method with the changed version. It is a good idea to make sure that MethodQueue definitions always contain updated methods, to avoid confusion between different versions of method files.

8.2 Editing MethodQueues

To edit an existing MethodQueue, open the MethodQueue icon with the right mouse button menu command, **Edit**. The **MethodQueue Editor** dialogue is displayed for the selected MethodQueue. **Edit MethodQueue** authorisation is required to edit a MethodQueue.

- To change the start condition or method name, select the line to be edited and click on the **Condition** or assigned system button respectively.
- To insert a new MethodQueue line after the currently selected line, click on **Insert**.
- To erase the currently selected line from the MethodQueue, click on **Delete**. In the dialogue box, check the system(s) for which the method is to be deleted.

8.3 Running a MethodQueue

Before starting a MethodQueue run, make sure that all systems used in the MethodQueue are connected with control mode connections (see Section 6.5) and are in **End** status. The system associated with the first **System** button in the MethodQueue definition must be connected to System control window 1, the second system to System control window 2 and so on. The MethodQueue will not start unless all required systems are connected in control mode.

To start a MethodQueue from the Main menu, select the MethodQueue icon and select **File:Run** or select **Run** from the right mouse button menu. The MethodQueue will start in accordance with the conditions defined in the MethodQueue setup. You cannot start or end a MethodQueue from the System control window.

Run MethodQueue authorisation is required to start a MethodQueue.

8.3.1 Method execution in MethodQueues

The start protocol for the first and each subsequent method step in the MethodQueue is displayed when the corresponding method is run. If you require unattended MethodQueue operation after the start of the first method step, make sure that subsequent method steps do not include a start protocol.

The **Condition** setting for each step in a MethodQueue determines the relative timing of the steps. If successive methods are run on the same system, the timing set in **Condition** applies from the completion of one method to the start of the next.

If successive methods are run on different systems, the Ready instruction in one method can be used to trigger the start of the next method. The Condition setting then applies from the Ready instruction to the start of the triggered method. This is useful for example in situations where a method on one system prepares the starting material for the next, and then continues to wash the system:

System 1 System 2

Apply sample

Elute

READY ----------Apply sample

Wash Elute

....

8.4 Displaying MethodQueues

Choose **MethodQueue:Display running** to display pending and running MethodQueues. A pending MethodQueue is one for which **Run** has been requested, but which has not yet started either because the system is not available or because the Setup time has not been reached.

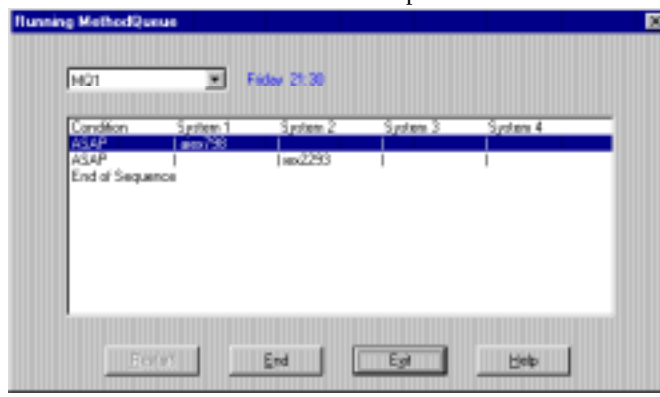


Figure 8-6. Displaying running and pending MethodQueues.

Use the list box at the top left corner of the dialogue to select a MethodQueue. The list shows pending and running MethodQueues.

For the selected MethodQueue, **Start** at shows the time when the MethodQueue is programmed to start. The actual time of start is shown for a currently running MethodQueue. The buttons in the MethodQueue display have the following functions:

Restart — Restarts the currently running MethodQueue if a start protocol has been terminated by Cancel.

End — Terminates a running MethodQueue after the current step. Any methods currently in operation will continue to run, and must be terminated with End in the System control window if they are not to run to completion. Clicking on End for a pending MethodQueue deletes the MethodQueue from the pending list.

Exit — Closes the MethodQueue monitor panel.

Introductory material

Methods and runs

Evaluation

System management

Appendices

9 Presenting results

A result file is automatically generated at the end of a run and contains a complete record of the run, including method, system settings, curve data and run log. The Evaluation module offers extensive facilities for presenting and evaluating curve data.

This chapter describes how to:

- view the documentation from a run
- present the chromatograms and curves of your result file
- compare chromatograms and curves
- print reports

9.1 Opening a result file

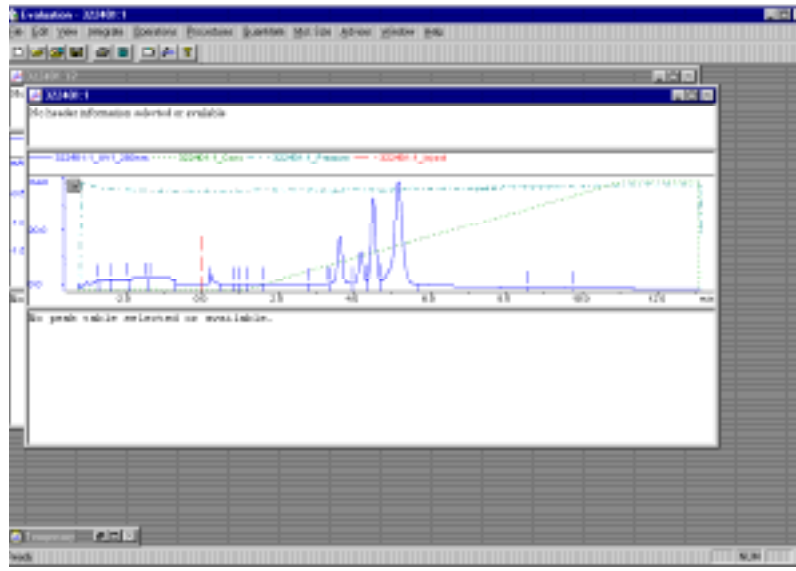


Figure 9-1. Evaluation module view.

To open a result file, either:

- double click on a result file icon in the **Results** window of the Main menu,
- select a result file icon in the **Results** window of the Main menu and select **File:Open**,

- click on the **Evaluation** icon in the Windows NT taskbar, select **File:Open** or click on the **Open** toolbar button, and select a result file from the **Open Result** dialogue,
- click on the **Evaluation** toolbar button in the Main menu and select a result file from the **Open Result** dialogue.

All contents of the opened result file are transferred to the Evaluation workspace. By default, the chromatograms in a run are shown as opened windows. The chromatogram window on top is the active window. There is also an iconised **Temporary** chromatogram window.

9.1.1 Chromatogram

A chromatogram includes a number of curves that have been created during a run, such as UV, conductivity, pH, fraction marks, etc. The original raw data curves cannot be deleted or modified, although they can be used as the basis for evaluation procedures and subsequent creation of new curves. A chromatogram also contains the curves created and saved during an evaluation session. The default name for the first chromatogram in a result file is 1.

9.1.2 Temporary chromatogram



The **Temporary** chromatogram is essentially an empty chromatogram and is specific to the Evaluation module. Thus, curves can be copied into **Temporary** using **Edit:Copy:Curve** and comparisons and/or evaluations can be performed. This is particularly useful if you do not want to clutter up your original chromatograms with a large number of curves. It can also be used to keep blank run curves or curves to compare when opening different result files. Information contained within the **Temporary** chromatogram is automatically saved from one evaluation session to the next, but is not saved within the result files. Click on the window restore button or select **Windows:Temporary**. The contents of the temporary chromatogram can be removed by selecting **Edit:Clear temporary chromatogram**.

9.2 Basic presentation of chromatograms

This section gives directions on how to access result files and optimise the presentation of a chromatogram and its curves via the so called **Chromatogram Layout** dialogue. The last evaluation operation that is performed can be undone using **Edit:Undo**.

9.2.1 The chromatogram window

The chromatogram window is divided into three main views for header information, run curves and peak tables. The displayed areas for the views can be adjusted by dragging the borders with the mouse cursor between the views.

Viewing the curves

The first time a chromatogram window is opened and viewed, a default layout is applied to display all of the original curves. The default layout can be changed by the user (see Section 9.2.9).

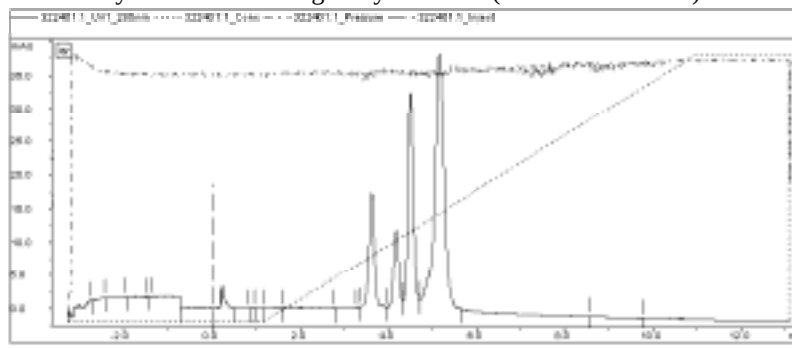


Figure 9-2. Displayed chromatogram in a newly opened result file.

Each curve is automatically assigned a default colour and style, with default information about each curve displayed in the key above the curves. This information includes the result file name, chromatogram name and curve name.

Each curve has a correspondingly coloured y-axis. To choose the appropriate y-axis scale, click on the y-axis until the desired scale is displayed or double click on the curve of interest.

Optimising the workspace

Chromatograms can be iconised in the desktop by clicking on the minimize button in a chromatogram window. Icons can be neatly arranged in the workspace by selecting **Windows:Arrange icons**.

To restore a window, click on the restore button for the iconised window or select the chromatogram name from the **Window** menu. You can also maximise a chromatogram window to fit in the whole Evaluation desktop by clicking on the maximize button.

To view several chromatogram windows side by side select **Windows:Tile**. Alternatively, **Windows:Cascade** will stack all of the open windows like a deck of cards.

9.2.2 Opening the Chromatogram Layout dialogue

Most of the changes that you are likely to make regarding chromatogram presentation, are made in the **Chromatogram Layout** dialogue. This is opened in one of two ways:

- Place the mouse cursor in the in the chromatogram window and select **Properties** from the right mouse button menu options. Note that the view from which you activate the **Properties** command determines the tab that is displayed in the **Chromatogram Layout** dialogue.
- Select **Edit:Chromatogram Layout**.

Note: You can apply any changes made in the **Chromatogram Layout** dialogue to all chromatograms by checking the **Apply to all chromatograms** option.

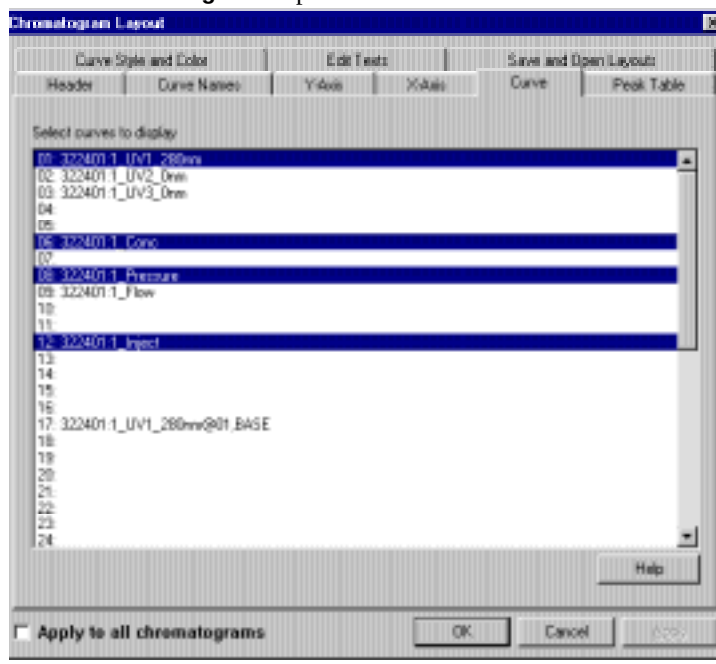


Figure 9-3. Chromatogram Layout dialogue, Curve tab.

The layout of the three views for header, curves and peak table can be modified in the various tabs that are displayed in the **Chromatogram Layout** dialogue. You can work freely in the **Chromatogram Layout** dialogue and all of the configurations are applied when you click on the **OK** button. If instead you want to close the dialogue without applying the changes you have made, click on the **Cancel** button. The main features of the **Chromatogram Layout** dialogue regarding chromatograms are described in the sections below. Features regarding peak tables are described in chapter 10.

9.2.3 Choosing the curve(s) you want to see

In the **Curve** tab of the **Chromatogram Layout** dialogue is a list of all curves contained within the chromatogram, numbered from 01 onwards. Select the curves you want to see in the chromatogram. Click on **OK** to return to the active chromatogram window.

9.2.4 Changing curve names

By default, names are sequentially built up from three components:

- result name,
- chromatogram name,
- curve name.

For example, a curve with the name 9139401:1_UV1_280, is derived from the result named 9139401. The chromatogram name is a number automatically given during a run, e.g. 1. The curve name corresponds to the curve type, e.g. UV1 for UV detection of an eluted component. If two or more curves of the same type were created within a result file, they will be numbered accordingly, e.g. UV1, UV2 etc. For systems using a variable wavelength detector, the wavelength for the UV curve is also given, e.g. 280.

If you do not want to display the entire names of the curves in both the dialogues and chromatogram windows:

1. Click on the **Curve Names** tab in the **Chromatogram Layout** dialogue.

2. Check the appropriate option boxes for the **Curve name appearance**.

3. Select the appropriate **Curve legend position** option.

It is usually sufficient to select the **Curve name** option if only one chromatogram is being evaluated. However, confusion may arise when more than one chromatogram is shown, so more complete names may be necessary.

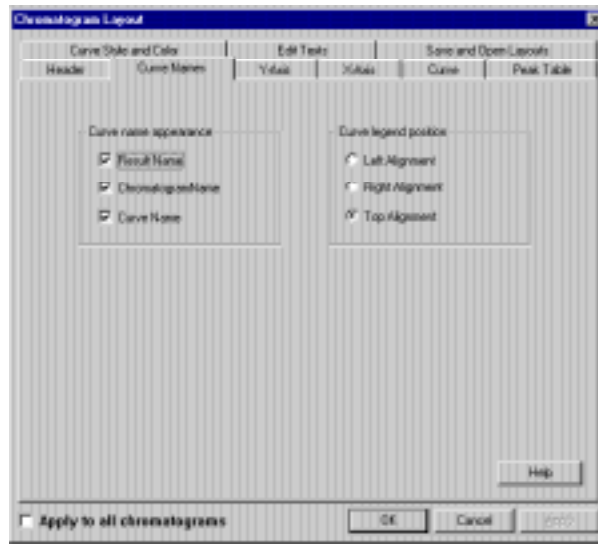


Figure 9-4. Curve Name dialogue.

9.2.5 Changing the colour and style of curves

All curves within a chromatogram are represented by a default colour and line style. Curves imported into the chromatogram or newly created curves, are automatically assigned a colour and line style.

To reassign the colour and/or style of a specific curve, do the following:

1. In the **Chromatogram Layout** dialogue, select the **Curve Style and Colour** tab.

2. To change the colour and/or line style of a curve, select the curve of interest from the list.

3. Select the desired colour and/or style.

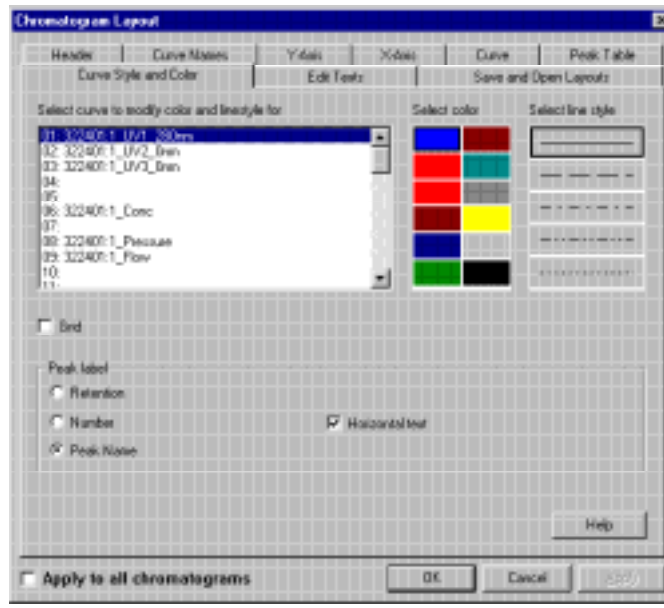


Figure 9-5. Line Style and Colour dialogue.

9.2.6 Changing and fixing the axes

By default, the y-axes are automatically scaled for each curve to show the whole curve. The x-axis scale is automatically displayed to show the whole run (ml or minutes).

It is possible to select 'fix', the minimum and maximum values for the axes of any curve and thereby select a specific part of the curve to be displayed.

Y-Axis

1. Click on the **Y-Axis** tab in the **Chromatogram Layout** dialogue.

2. Select the appropriate curve from the list for which you want to fix the scale. Click on the **Fixed** option.

3. Type in the desired minimum and maximum values for the y-axis. If you click on **All with this unit**, other curves that have the same y-axis units as the current scaled curve will be similarly scaled. Click on **OK**.

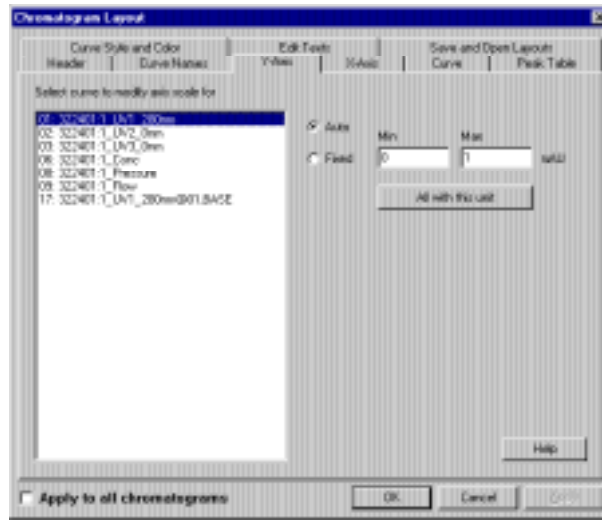


Figure 9-6. Chromatogram Layout dialogue, Y-axis tab.

Note: **All with this unit** will only be applied to existing curves. It will not be applied to new curves created after this function was last used. New curves are automatically scaled.

X-Axis

1. Click on the **X-Axis** tab in the **Chromatogram Layout** dialogue.

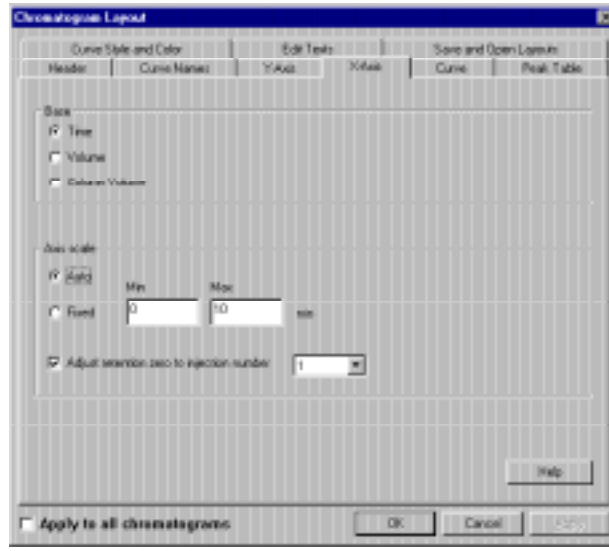


Figure 9-7. Chromatogram Layout dialogue, X-axis tab.

2. Select the appropriate choice from the **Base** field, either **Time** of retention, **Volume** or **Column volume**.

Note: Some calculated curves, e.g. baselines, exist in only one base and may seem to "disappear" when the base is changed.

3. Click on the **Axis Scale**, **Fixed** option. Type in the desired minimum and maximum values for the x-axis.

4. If desired, select (check) **Adjust retention zero to injection number**. This function sets the time/volume to zero at the injection mark, i.e. when the sample was injected onto the column. The time and volume before injection will become negative values. The injection mark to be used as zero is denoted by the number.

Note: Adjust retention zero does not affect the log book.

9.2.7 Viewing information about the run

You may wish to display header information at the top of a chromatogram detailing the variables, scouting variables, questions and/or notes. Header information cannot be displayed for imported chromatograms.

1. In the **Chromatogram Layout box**, click on the **Header** tab.

2. Check the options to be included in the header of the chromatogram window.

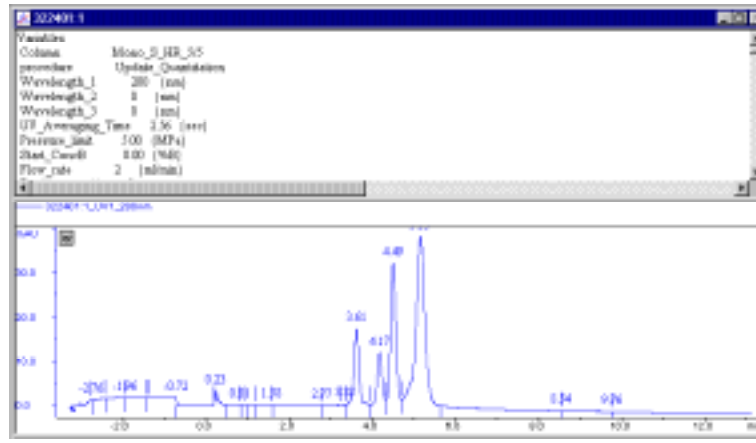


Figure 9-8. Chromatogram with header information displayed.

9.2.8 Saving and applying a layout

All configurations that you make in the **Chromatogram Layout** dialogue can be saved as a layout. It is possible to apply saved layouts to other chromatograms. All saved layouts are user specific.

To save a layout

1. Open the **Chromatogram Layout** dialogue and make the appropriate layout configuration within the various tabs. Note that you can return to the chromatogram window by clicking on **OK** to see the applied affects of a given configuration and return again to the **Chromatogram Layout** dialogue to perform further changes.

2. Select the **Save and Open Layouts** tab and click on **Save current layout as**.

3. Enter a name for the layout in the displayed dialogue. If you want the current layout to be the new default layout, check the **Save as default** option.

4. Click on **OK** to save the layout. The new name is added to the **Saved Layouts** list.

To apply a layout

1. Select the **Save and Open Layouts** tab.

2. Select a layout from the **Saved Layout** list and click on the **Open selected layout** button. The layout is automatically applied to the active chromatogram window. If the same layout is to be applied to all chromatograms on the Evaluation workspace, select (check) the **Apply to all chromatograms** option.

9.2.9 Viewing a grid in the chromatogram window

You can display a grid in the chromatogram window:

1. In the **Chromatogram Layout** dialogue, select the **Curve Style and Colour** tab.

2. Check the **Grid** option.

To remove the grid uncheck the **Grid** option.

9.3 Other presentation possibilities

The Evaluation module allows you to perform operations on the curves to optimise the presentation.

9.3.1 Showing part of a curve

This section deals with the selection of just part of a curve for purposes of closer examination of details and for presentation. This can be done in three different ways:

- magnification using the zoom function
- fixing the axes
- cutting the curves

The zoom function

In the active chromatogram window, it is possible to zoom in on a designated area of the chromatogram. This is the easiest and quickest way to enlarge different parts of a curve.

1. Place the mouse pointer in any corner of the intended area to be magnified.

2. Press and hold the left mouse button. A magnifying-glass icon will replace the mouse pointer arrow on the screen.

3. Drag out a box from the point of origin to cover the area to be magnified. Release the mouse button.

   The selected region is now displayed in the entire chromatogram window, together with appropriate scales for the y- and x-axes.

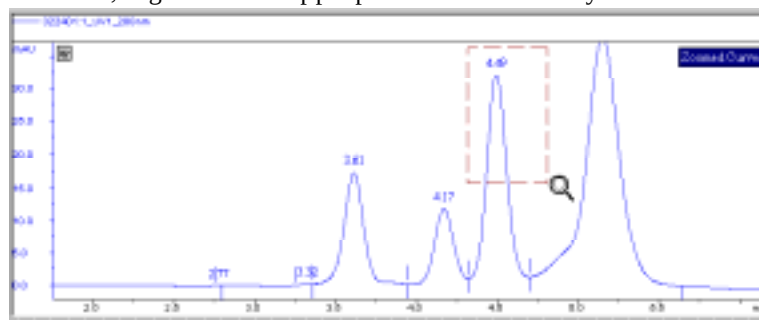


Figure 9-9. Illustration of the chromatogram Zoom function.

4. To move around in the chromatogram at the current zoom scale, use the cursor-arrow keys on the keyboard.

5. To undo the last zoom step, select **Undo zoom** from the right mouse button menu. To reset all zoom steps at once, i.e. no zoom applied, select **Reset zoom** from the right mouse button menu.

Alternatively:

Use the <Page Down> and <Page Up> keys to zoom in and zoom out respectively on the whole chromatogram.

Fixed scale axes

Another way to display only part of a curve is to choose, or 'fix', the minimum and maximum values of the y- and/or x- axes in the **Chromatogram Layout** dialogue (see Section 9.2.6).

Cutting curves

The cut curve function allows a region of the curve between two values on the x-axis to be cut and stored as a new curve. This is done in the following way:

1. Select **Operations:Cut curve**.

2. In the displayed dialogue select the curve(s) to be operated on. Click on **OK**.

3. The selected curve will now be shown in a new window which also contains two vertical cursor lines. To facilitate the cutting process, it is possible to use the zoom function within the window.

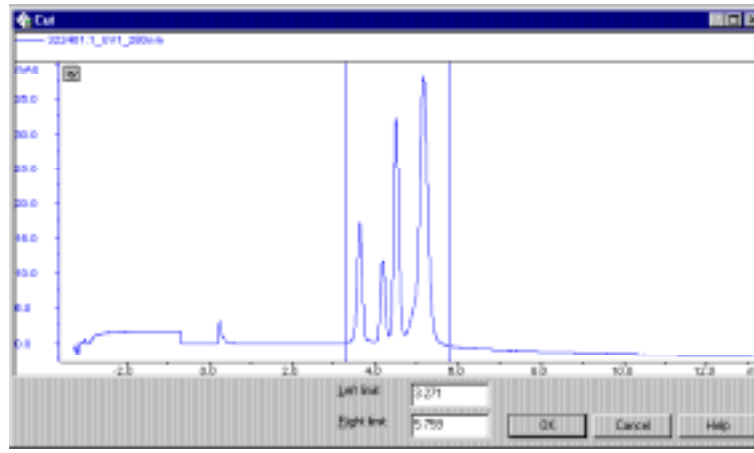


Figure 9-10. Cut window.

To select the region to be cut, either:

- drag the two cursor lines to define the left and right limits of the cut area, or,
- type the desired left and right limit values in the boxes marked **Left limit** and **Right limit**.

Note: The areas outside of the **Left limit** and **Right limit** will not be saved in the newly created cut curve. Thus, the x-axis of the new saved curve will not begin at zero unless designated as one of the limits. The original curve is not changed.

4. Click on **OK**. A new dialogue is displayed. Select whether to save the new cut curve in the **Source chromatogram**, i.e. the current active chromatogram, or in a **New chromatogram**. If you select the latter option, you can change the name of the chromatogram. Click on **OK**.

5. The original source chromatogram is displayed. If the destination of the cut curve was the source chromatogram, the cut curve is automatically displayed. If the destination of the cut curve was a new chromatogram, this will be represented as a new, open chromatogram window. Restore the iconised window to open the chromatogram window containing the cut curve.

9.3.2 Reducing noise and removing ghost peaks

Sometimes the chromatograms may contain curves with a noisy baseline. The noise can be caused by several things e.g. a dirty flow cell, air bubbles, electrical noise, dirty buffers etc. The amount of noise can usually be reduced by taking proper precautions, e.g. filtration of buffers and instrument maintenance.

Smoothing a curve

The smoothing function allows background noise to be reduced or removed from any selected curve. The type of smoothing function you should choose depends upon the type of noise encountered.

1. Select **Operations:Smooth**.

2. Select the source curve to be smoothed and its target destination. By default, smoothed curves are given the suffix, **SMTH**.

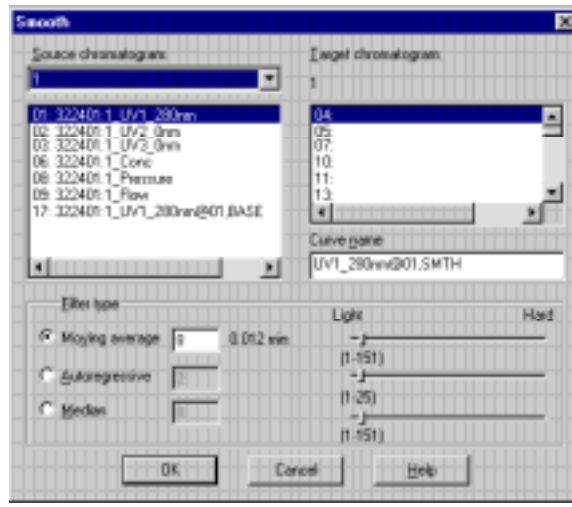


Figure 9-11. Smooth dialogue.

3. Select the **Filter type** to be applied in the smoothing operation. This selection can be based on the following criteria:

   - Choose **Moving average** if you have noise along most of the curve.
     Smoothing with this filter affects peak height but not retention. There is little effect on peak area.
   - Choose **Autoregressive** if you have periodic noise along the whole curve.
     Smoothing with this filter will affect peak height and retention, although has little affect on peak area.
   - Choose **Median** if there is only one or a few noise spikes, e.g. caused by air bubbles, or if the noise is confined to only a small part of the curve.
     Smoothing with this filter may give flattened peaks and affect peak areas slightly but will not affect the retention.

4. Select an appropriate smoothing parameter value from **Light** to **Hard** for the selected filter. The smoothing effect increases with increasing parameter values.

   Smoothing is always a compromise between noise removal and preservation of peak shape. The easiest way to find the optimum smoothing effect is to start with a low parameter value, e.g. the default value, and increase it until the best result is achieved. A useful strategy is to increment the parameter value by the default value for each try.

5. Click on **OK**.

The formulae for the filters are described in Appendix D.1.

9.3.3 Subtracting a blank run curve

This is a frequently used function in presentations, especially if the curves have a drifting baseline or "ghost" peaks.

Note: If the ghost peaks come from impurities in the eluents, all equilibration of the columns should be the same from run to run. If, for example, the equilibration volume with buffer A is larger before a blank run curve than before a separation, your ghost peaks might be higher in the blank run curve.

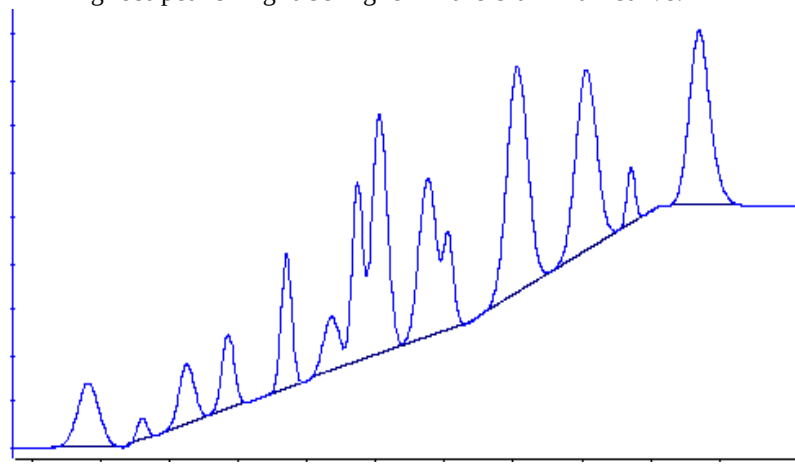


Figure 9-12. UV curve with baseline prior to subtraction of the baseline.

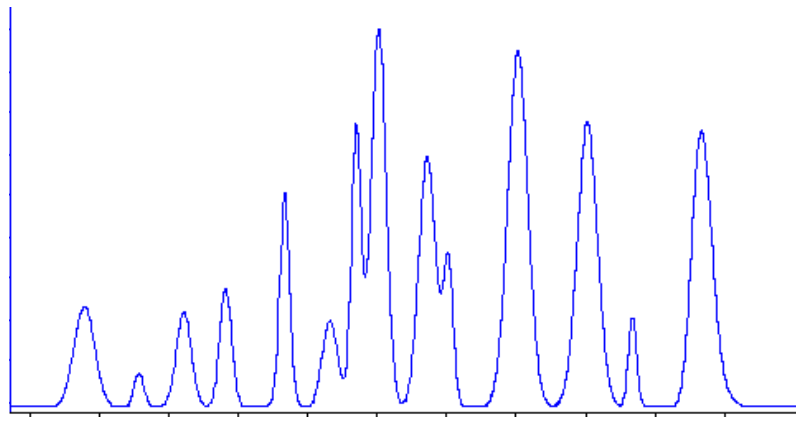


Figure 9-13. UV curve after subtraction of the baseline.

Alternative A: Importing a blank run curve

If a blank run curve was done, this may have been stored in another result file. To access the blank run curve:

1. Ensure that the destination chromatogram has been opened and is the active window on the workspace.

2. Select **File:Open** and then from the select **Curves** from the menu cascade. The **Open Curves** dialogue is displayed.

3. Locate and double click on the result file containing the blank run curve. The curves in the first chromatogram are displayed.

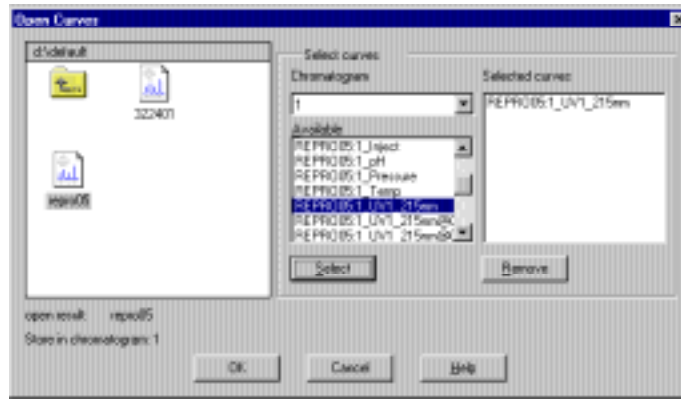


Figure 9-14. Open Curves dialogue with a result file selected.

4. Select the curve corresponding to the blank run curve and click on the **Select** button. The selected curve will now be displayed in the **Selected curves** list.

   To remove a curve from the list, select it and click on **Remove**.

   If there is more than one chromatogram in the result file and the blank run curve resides in another chromatogram, select the appropriate chromatogram from the drop-down list. The curves for that chromatogram are displayed from which you should make the appropriate selection.

5. To import the curve click on **OK**.

Note: For more detailed information about how to import curves, chromatograms and other results, see Section 9.4.

Alternative B: Calculating a baseline

If there is no blank run curve, you can instead create a baseline with **Integrate:Calculate baseline** (see Section 10.1.1).

Subtracting the blank run curve

Select **Operations:Subtract** to subtract the blank run curve or the baseline away from the sample curve. Click on **OK**. All resulting curves from the subtract operation receive the SUB suffix.

9.3.4 Adding curves

In some runs, several sequential chromatograms may have been created, for example, when the instruction **New chromatogram** has been used in the method, thus creating different chromatograms during the run. In order to view and evaluate the resultant curve of all the chromatogram parts, the curves must be added together. The common situation is when you have a number of chromatograms within the same result file and you want to add the curves. In some circumstances, curves may need to be imported from other result files.

To add curves:

1. Select and view the first chromatogram in the sequence.

2. Select **Operations:Add**. Add the first curve in the sequence to the second curve in the sequence from the appropriate chromatograms.

3. Add the result of the previous step to the next curve in the sequence.

4. Repeat this process until all curves have been added together. The final curve should be the cumulative curve for the whole run. All curves created using the **Add** operation receive the ADD suffix.

9.3.5 Entering text in the chromatogram

Basic annotations can be added to the chromatogram.

1. Place the mouse pointer in the curves view of the chromatogram window and select **Add text** from the right mouse button menu. Alternatively select the **Edit:Text:Add** command. The mouse pointer is replaced with an **ABC** pointer.

2. Position the pointer where you want to insert text in the chromatogram and click the left mouse button once.

3. In the dialogue that appears, type the desired text and then click on **OK**. Now the text can be viewed on the chromatogram. The text is saved at the position where it is placed in the chromatogram window and is not linked to any curve. Text cannot be moved within the window once it has been placed.

If you want to edit or delete an inserted text:

1. Open the **Chromatogram Layout** dialogue and select the **Edit Texts** tab. Alternatively, select the **Edit:Text:Edit** command and the **Edit Texts** tab is displayed automatically.

2. Select the specific text that you want to edit and make the appropriate changes in the **Selected text** field. Click on **Change text**.

3. Select the specific text that you want to delete and click on **Delete text**.

4. Click on **OK** to close the dialogue and apply the changes.

9.3.6 Pooling fractions

During a separation fractions are sequentially collected, with each fraction containing a set volume of sample. Each fraction is numbered according to its order in the sequence and this information is saved as a curve under the name, Frac. Selection of this curve in the **Chromatogram Layout** dialogue will display the precise contents of each fraction relative to the information displayed on the UV detection curve.

Since the peak of interest may not always be collected in one fraction, it may be desirable to pool some fractions.

To pool the fractions:

1. Select **Operations:Pool fractions**. The **Frac curve** is automatically displayed for the active chromatogram. The chromatogram selection can, of course, be changed.

2. In the **Fraction no.** field, select the fractions to be pooled.

3. Select the destination for the new curve. The default name of the new curve has POOL as its suffix.

3. Click on **OK**.

The active chromatogram will now display both the original and pooled fraction curves, so deselect the original fraction curve in the **Chromatogram Layout** dialogue.

9.3.7 Renaming chromatograms, curves and peak tables

Sometimes, it may be desired to change the name of a chromatogram, curve or peak table. To do this, close the **Chromatogram Layout** dialogue and then:

1. Select **Edit:Rename** and the relevant menu cascade option **Chromatogram**, **Curve** or **Peak Table**.

2. Select the appropriate object in the displayed dialogue and type in the new name. Click on **OK**. The new name will replace the old one rather than creating a new curve or chromatogram.

Note: The original raw data curves cannot be renamed and are not therefore given as options.

9.4 Comparing different runs

The previous sections dealt with the manipulation of single curves within a chromatogram. The following sections describe how to make comparisons between two or more curves or chromatograms from different runs and detail how best to present them.

It is possible to:

- view several chromatograms at the same time
- overlay curves from different runs in one chromatogram
- stack curves from different runs in one chromatogram
- stretch curves to make comparisons easier
- create mirror images

9.4.1 Comparing chromatograms from different runs

To import chromatograms from other result files into an already opened result file, two functions can be used, namely **File:Open to compare** or **File:Open**. The former option is most useful in searching for many chromatograms in a specific folder based on defined selection criteria. The latter option is best used to import any individual chromatograms from result files in different folders. The imported chromatograms are sequentially numbered (11, 12, 13, etc.) for identification purposes. Up to 10 chromatograms can be made available at the same time on the evaluation workspace.

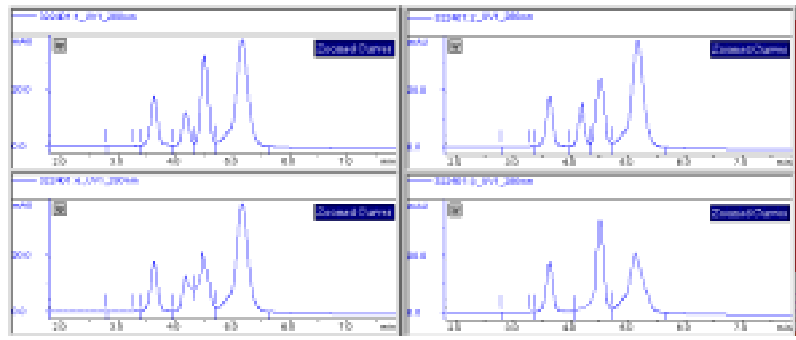


Figure 9-15. Windows:Tile function to display many chromatograms.

Alternative A: Import chromatograms using Open to compare

This method is useful, for example, when importing chromatograms from all files of a scouting folder.

1. Click on **File:Open to compare:Chromatograms**. The **Open Chromatograms to Compare** dialogue is displayed.

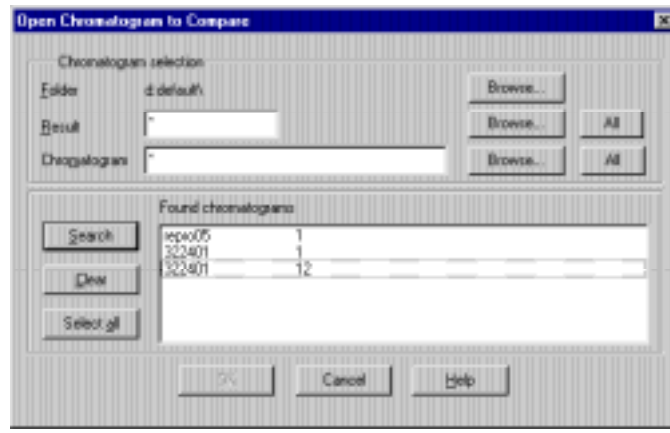


Figure 9-16. File:Open to compare chromatograms dialogue.

2. The search will take place in the displayed folder only. To select another folder, click on the **Browse** button and open the desired folder.

3. The search for chromatograms will take place in all result files within the selected folder as denoted by the asterisk '*****'. You can instead select a specific result file using the **Browse** function. Moreover, you can use wildcard characters to search within result files with a specific name profile.

You can use standard wildcard characters in the file name specification (* stands for any number of characters and ? for any single character). For example:

iex	will search files named iex
iex*	will search all files with names beginning iex
*iex	will search all files with names ending iex
?iex	will search only 4-character names ending in iex

To return to the default setting to search in all result files, click on **All**.

4. Click on the **Search** button and a list of chromatograms will be displayed based on the designated search criteria. A new search can be performed with new search criteria without erasing the first found chromatograms from the list.

5. Select the chromatograms that you want to import. If you click on the **Select All** button, all of the displayed chromatograms are selected for importing. If you want to clear the list of displayed chromatograms, click on **Clear**.

6. Click on **OK** and all selected chromatograms are shown on the Evaluation workspace.

Alternative B: Importing using Open

1. Select **File:Open:Chromatogram**.

2. Select the desired result file by double clicking on it, and all of the chromatograms contained within will be displayed. Normally it is only one chromatogram and is named "1".

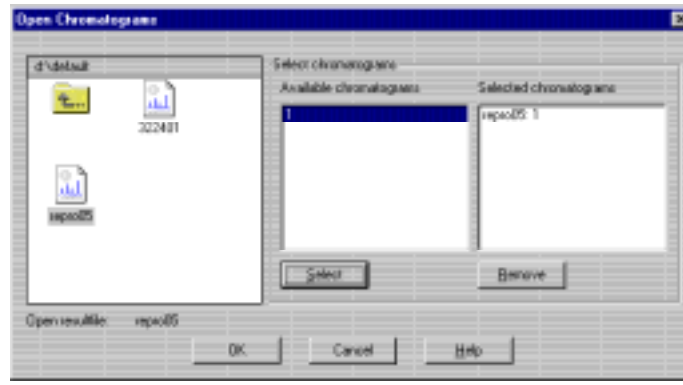


Figure 9-17. File:Open chromatogram dialogue.

3. Select the chromatogram(s) of interest and press the **Select** button. Selected chromatograms are added to the **Selected chromatograms** list. Chromatograms can be deselected by using the **Remove** command button.

4. Repeat steps 2-3 for chromatograms in other result files.

5. Click on **OK**.

Viewing all chromatograms

1. Double click on the imported chromatogram icons to open them.

2. Simultaneously display the chromatograms by selecting **Windows:Tile** or layer them by selecting **Windows:Cascade**.

3 Chromatogram windows can be individually sized and the presentation of the curves changed.

4. If you want to have the same scale on all of the chromatograms, open the **Chromatogram Layout** dialogue for any chromatogram, make the changes and select (check) the **Apply to all chromatograms** option.

   Imported chromatograms cannot be shown with column volume as the x-axis base.

9.4.2 Comparing curves

Curves from different runs can be imported or copied into one chromatogram for comparison.

Alternative A: Importing curves using Open to compare

Result files contained in the same folder can be automatically searched to locate all curves of a specified type, for example, all UV curves. This is especially useful for comparison of curves from scouting runs. Moreover, the imported curves can be automatically overlaid, stacked or be presented as mirror images.



1. Select **File:Open to compare:Curves** or click on the **Open curves to compare** toolbar button. Select the search criteria for the folder, result, chromatogram and/or curve name using the respective **Browse** command buttons.

   Wildcard characters, * and ?, can also be used to further specify the search parameters (see Section 9.4.1 Alternative A).

The UV curves are identified with number and sometimes wavelength. For example, UV1_280, UV2_280 and UV1_254 are all different curve types. To search for all UV curves, enter UV* in the **Curve name** text field.

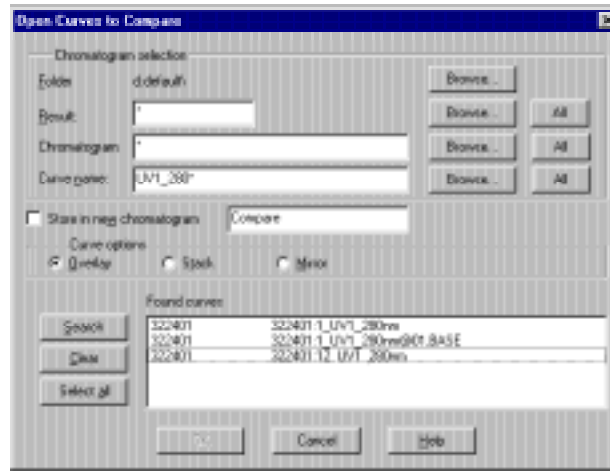


Figure 9-18. Open Curves to Compare curves dialogue.

2. Click on **Search** and a list of found curves will be displayed based on the designated search criteria. A new search can be performed with new search criteria without erasing curves located in the previous search.

3. Select the curves that you want to be imported. Click on **Select All** if you want to import all of the curves.

4. You can import the curves into a new chromatogram by selecting (checking) the **Store in new chromatogram** option. This is recommended to keep the source chromatogram free of too many additional curves.

5. Select how the imported curves will be displayed by clicking on one of the buttons; **Overlay**, **Stack** or **Mirror**.

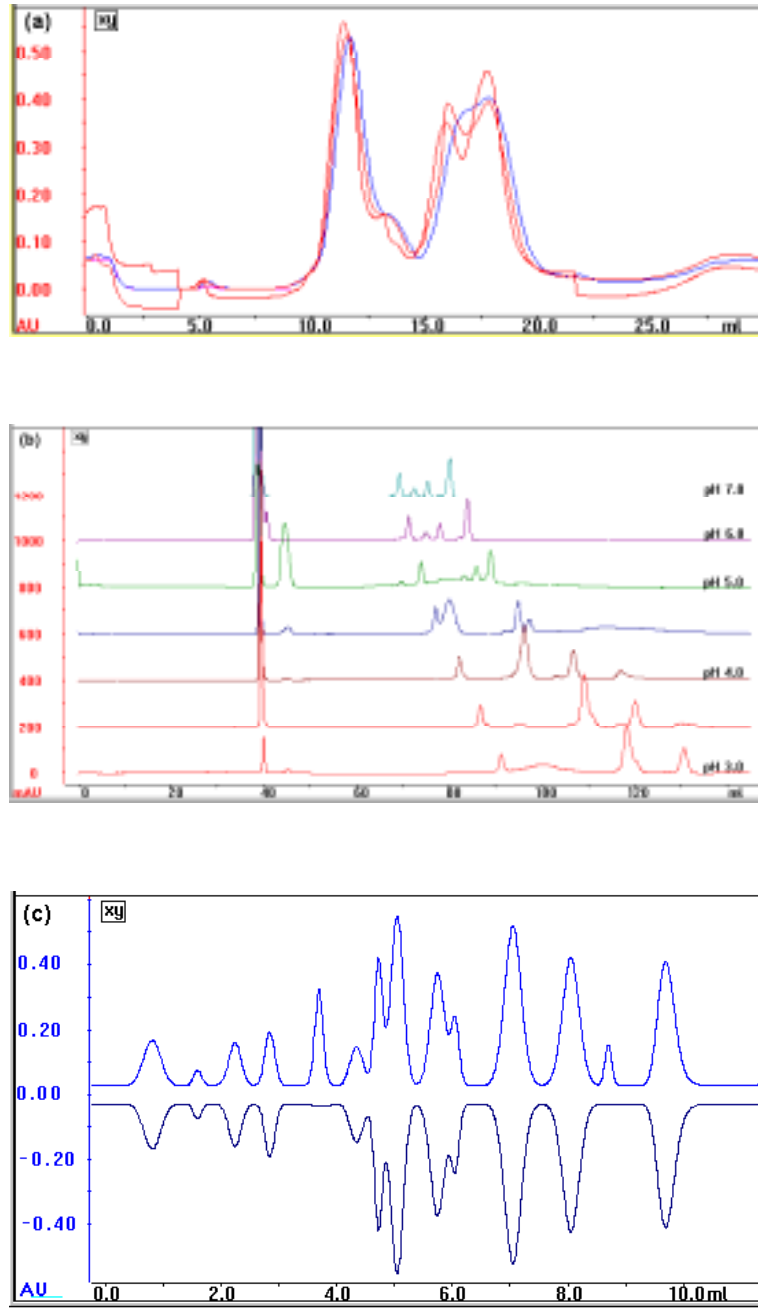


Figure 9-19. Different presentation options for comparison of imported curves; (a) overlaid curves, (b) stacked curves, (c) mirrored curves.

Overlay This presents the imported curves overlaid one on another.

Stack This presents the imported curves with a given offset y-axis value so that the curves are stacked and distinct from one another.

Mirror This should be ideally used to view two imported curves. One curve is inverted in the y-axis and thus appears to mirror the other curve.

6. When you have made you selection, click on **OK**.

   If you selected the **Stack** option (see step 5.), the **Stack Offset** dialogue is displayed. You can change the displayed value to increase or decrease the offset distance between the curves. If the selected curves have different y-axis units, the dialogue is displayed for each curve. Click on **OK**.

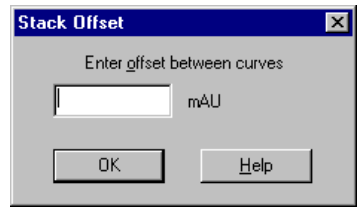


Figure 9-20. Stack Offset dialogue.

7. Imported curves are displayed in either the source chromatogram or in a new chromatogram that you created. In the latter case, new chromatograms are displayed as iconised windows. Restore the window(s) to view the imported curves.

8. Select the curves that you want to view in the **Chromatogram Layout** dialogue. Curves can also be scaled individually or all with the same scale using the **All with this unit** function in the **Chromatogram Layout** dialogue (see Section 9.2.6).

   If you stacked the curves and you want to change the stack offset, the easiest way is to import the curves again with another offset value. The individual curves can also be moved (see Section 9.4.3).

Alternative B: Importing curves using Open

Using the **File:Open:Curves** function, individual curves may be imported into the active chromatogram.

1. Ensure that the destination chromatogram for the imported curve(s) is active on the screen.

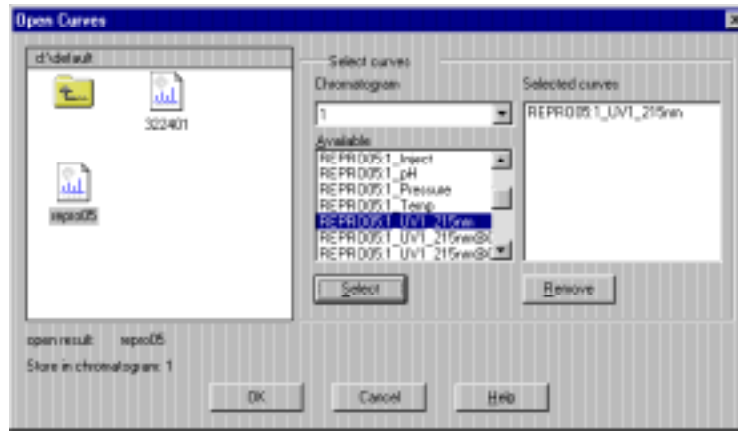


Figure 9-21. Open curves dialogue.

2. Select **File:Open**. The **Open Curves** dialogue is displayed.

3. Select the folder in which to search for curves.

4. Click on the result file of choice and, where appropriate, the specific chromatogram containing the desired curve. The chosen chromatogram and curves contained therein will be listed.

5. Select the desired curve and click on the **Select** button. The selected curve will now be displayed in the **Selected curves** list.

6. If you want to choose more curves from other chromatograms repeat steps 3-4. When all the desired curves have been selected, click on **OK**.

7. Restore the chromatogram window and open the **Chromatogram Layout** dialogue. Select the curves that you want to view. Curves can be scaled individually or all with the same scale using the **All with this unit** function (see Section 9.2.6).

Alternative C: Copying curves into one chromatogram

Curves can be copied between chromatograms present in the Evaluation desktop. For effective comparison of curves, it is suitable to transfer all relevant curves to a single chromatogram. This is best achieved by:

- creating a new chromatogram using **File:New: Chromatogram**, and copying curves into it from other chromatograms, or,

- copying an existing chromatogram using **Edit:Copy:Chromatogram** and importing more curves into it, or,
- copying curves into the **Temporary** chromatogram (see Section 9.1.2). You can perform evaluations in the **Temporary** chromatogram and transfer the final curves to other destination chromatograms. The unwanted contents remaining in the **Temporary** chromatogram can then be removed using **Edit:Clear temporary** chromatogram.

To copy curves:

1. Select **Edit:Copy:Curve**.

2. Select the source chromatogram and the curve of interest. Select the target chromatogram. Click on the **Copy** button to effect the copy. Stay within the same dialogue to repeat this step for as many other curves you want, from the same or different chromatograms. When you have copied all desired curves, click on **Exit**.

3. Open the destination chromatogram and access the **Chromatogram Layout** dialogue. Select in the **Chromatogram Layout** dialogue the curves that you want to view. Curves can be scaled individually or all with the same scale using the **All with this unit** function (see Section 9.2.6).

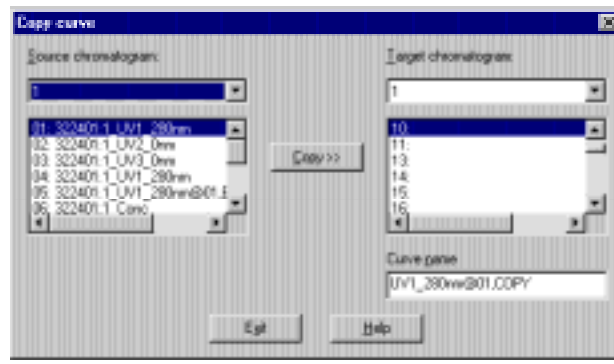


Figure 9-22. Edit:Copy curve dialogue.

9.4.3 Stacking and stretching curves

Several tools are available to stack and stretch curves from different runs to better visualise the differences. These tools are normalising curves, shifting curves and stretching curves. These allow you to manually reproduce the **Stack** and **Mirror** functions associated with the **Open to compare:Curves** operation (see Section 9.4.2 Alternative A), and more besides. If the curves have been stacked with the **Open to compare curves** operation and you want to change the stack offset, the

easiest way is to repeat the operation with another offset. The curves can also be stacked and stretched individually as described below. The operations presented below all require the curves to be present in one chromatogram (see Section 9.4.2).

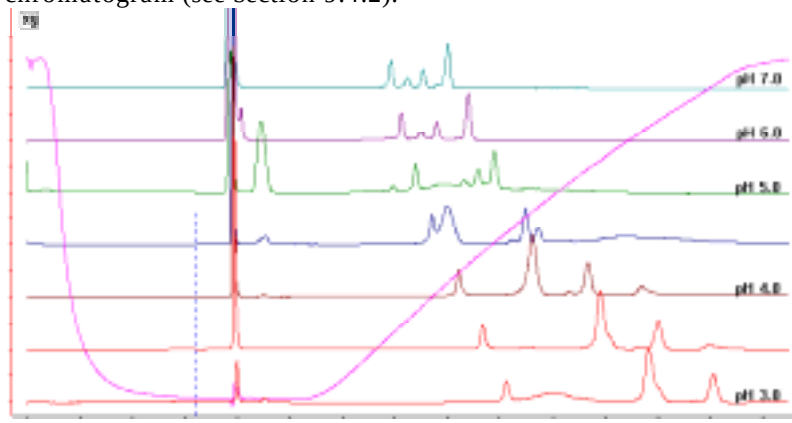


Figure 9-23. Stacking curves.

Alternative A: Stacking and stretching curves using the normalise function

The simplest method to align curves with respect to the x-axis or the y-axis for easier visualisation, is to use the normalise function.

To select the curve to be moved within a chromatogram:

1. Select **Operations:Normalise**. The **Normalise** dialogue is displayed.

2. Select the curve to be normalised and a reference curve to be normalised against. For example, if you want to stack curves, select the curve at the bottom of the stack to be normalised against and the curve to be moved as normalised. Click on **OK**.

3. The **Normalise** window is displayed. A box surrounds the curve selected to be normalised.

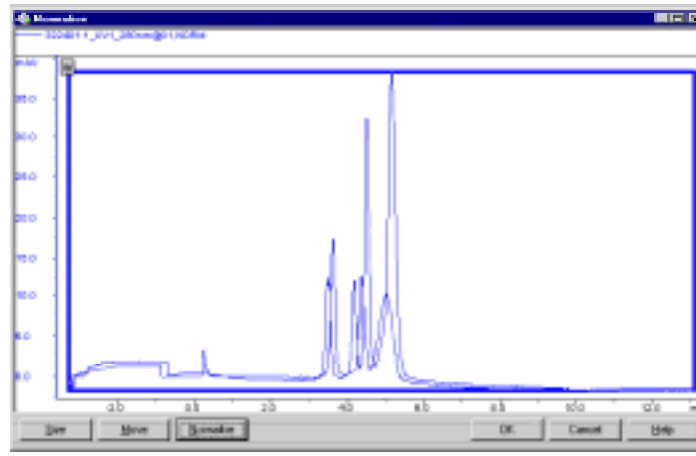


Figure 9-24. Normalise window.

You can now use the following functions:

Size Allows the selected curve to be stretched along its y-axis or x-axis. Click on **Size** and then drag the coloured box either along its y-axis or x-axis. This is useful for comparison of curves with, for example, different gradient lengths.

Move Allows the selected curve to be moved to any position on the chromatogram. Axes are automatically re-scaled to accommodate the new positioning. This function is useful for stacking curves. Click on **Move** and then move the curve with the mouse pointer. Click on the mouse button when the curve is in the correct position.

Normalise The curve to be normalised will be adjusted to the help curve. Thus, the height of the highest peak on both curves will be the same and will occur at the same retention point. The curve to be normalised is automatically moved along the x-axis and stretched along the y-axis.

4. When all operations have been performed, click on **OK** to save the new normalised curve. Open the **Chromatogram Layout** dialogue to select the normalised curve for viewing.

5. Repeat the procedure for all curves you want to stack or stretch.

Alternative B: Moving a curve using the Shift function

If more precise positioning of curves is required, then the shift function should be used. This function is similar to **Normalise:Move** except that each curve is repositioned by a precise value instead of by eye and the instruction logged in the evaluation log.

1. Select **Operations:Shift**. The **Shift** dialogue is displayed. Select the curve to be shifted.

2. Select the axis along which the shift is to be made, i.e. along the x-axis (**Shift retention**) or the y-axis (**Shift amplitude**). Enter the shift value and click on **OK**.

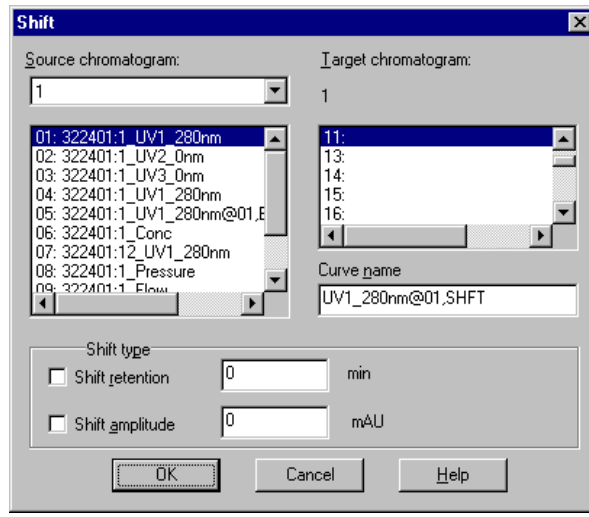


Figure 9-25. Shift dialogue.

Alternative C: Stretching and shrinking a curve using multiply

Curves can be stretched or shrunk in the x or y plane using the multiply function. This function is similar to **Normalise:Size** except that each curve is repositioned with precise numbers instead of by eye and the instruction logged in the evaluation log.

1. Click on **Operations:Multiply** and select the curve to be multiplied.

2. Select (check) the appropriate axis for multiplication, either **Multiply retention** and/or **Multiply amplitude**.

3. Insert the appropriate multiplication factor and click on **OK**.

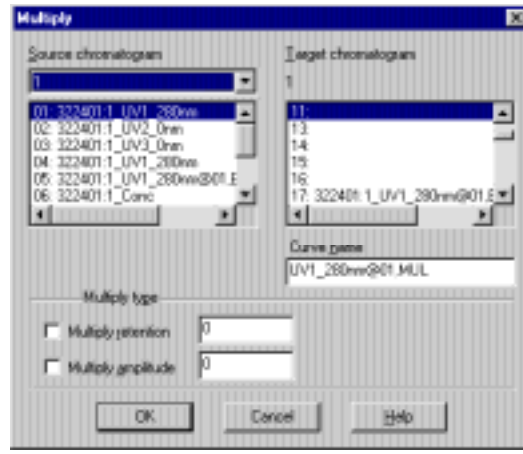


Figure 9-26. Multiply dialogue.

9.4.4 Mirror images of curves

A very useful way of comparing the features of two curves is to produce a mirror image of one curve. To achieve this:

1. Select **Operations:Multiply**.

2. Select the desired curve to be mirrored and select **Multiply amplitude** in the **Multiply type** field.

3. Type in a multiplication integer of -1 and click on OK.

4. Shift the mirror image curve downwards for an improved presentation (see Alternative B above).

Now the mirror image of the original curve will be displayed in the active window. Select/deselect for the other curves wanted in the active chromatogram window in the **Chromatogram Layout** dialogue.

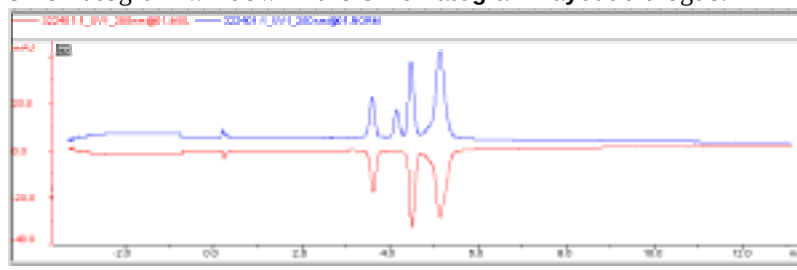


Figure 9-27. Two curves displayed in mirror image.

9.4.5 Matching protein activity to a curve

The Evaluation operations allow you to combine data from the results of protein activity assays, for example ELISA, with the data contained on the UV curve. A presentation comparing the activity curve with the UV curve can then be made.

1. Select **Operations:Activity histogram**. A dialogue is displayed with the fraction curve for the specific chromatogram already selected. Change the source and target chromatogram selection if desired.

2. All the component fractions of the fractions curve are listed. For each fraction you can type in the corresponding activity value. When all values have been entered, click on **OK**.

9.5 Saving results

Any changes to chromatograms, including all new created curves and all imported or created chromatograms, can be saved in either of two ways, using:

- **File:Save** or the **Save** toolbar button, which saves all changes in the original result file,
- **File:Save as**, which allows you to create a new result file in the specified target folder.

Note: All curves created during the manipulations will also be saved. This may not always be desirable. Before saving, remove unwanted curves from a chromatogram using **Edit:Delete:Curve**. The original curves can never be deleted.

9.6 Printing active chromatograms

To print out the active chromatogram select **File:Print** or click on the **Print** toolbar button. If you want to print out several chromatograms ensure that these are open on the workspace before selecting **File:Print**.

A dialogue appears allowing you to select the print format of the chromatograms. Alternatively, you can click on the **Other** option and a dialogue appears that allows you to select the number of chromatograms in each column and row to be used in the printed document.

Click on **OK** to print out the chromatograms.

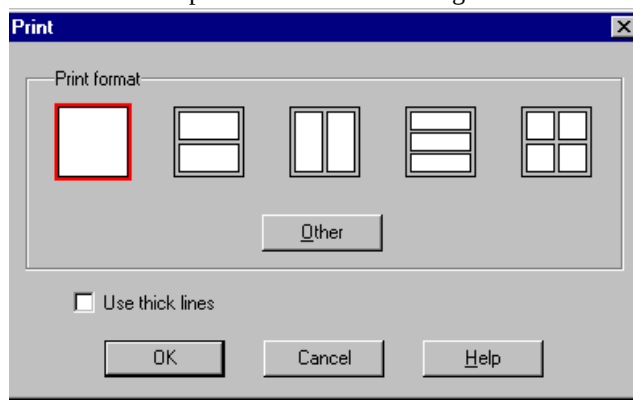


Figure 9-28. Print dialogue.

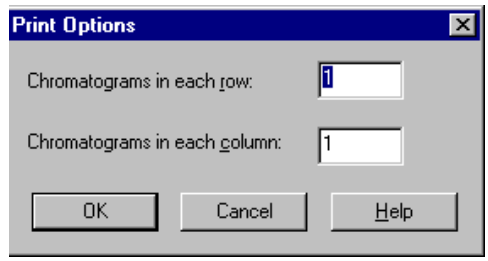


Figure 9-29. Print Options dialogue.

If the Documentation is open, click on the **Print** button to print any components in the documentation.

9.7 Printing reports

A printed record of all documentation concerning a run, including the method and Run Log, can be obtained as follows.

1. Select **File:Report** or click on the **Report** toolbar button and the **Generate Report** dialogue is displayed. If you have defined report formats, you can choose one and then press **Print**.

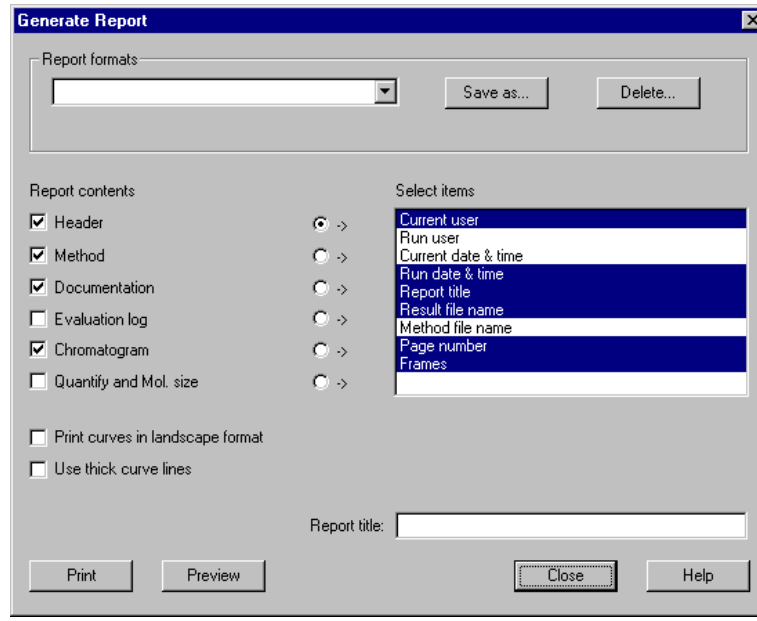


Figure 9-30. Generate Report dialogue.

2. Select the main items to be included in your report by placing a check mark beside each of the relevant options under **Report contents**.

3. Associated with each report content item is a radio button. Selection of a radio button lists a subset of items linked to the specific report contents option. Select the items which are to be included in the report.

   One of the report options, **Chromatogram**, is described in more detail (see below).

Note: It is possible to press a radio button and select items even if the corresponding report option is not selected (checked). Information will only be included in the report if the option under **Report Contents** is selected.

4. When you have decided on the report contents and format, you can preview the report by clicking on **Preview**. You can choose to print the curves in landscape view by selecting (checking) that option. If you want to print out the curves with thicker lines, for example to produce overhead transparancies, click on the **Use thick curve lines** option. To print the report click on **Print**.

Chromatogram

Selection of the radio button associated with the report content, **Chromatogram**, allows you to specify which chromatograms and/or chromatogram components are to be included in a report.

1. Click on the radio button for **Chromatogram**.

2. Make a selection from the item list:

Current chromatogram	The chromatogram that is currently active on the screen.
All chromatograms	All chromatograms on the evaluation desktop, including those that are shown as icons
1,**2**,...etc.	A specifically named chromatogram.

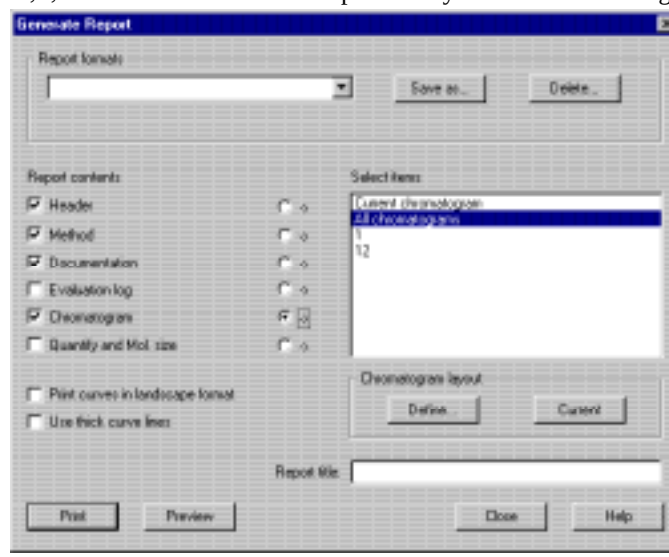


Figure 9-31. Selection of chromatogram content in the Generate Report dialogue.

3. If you want the chromatogram(s) to be printed out as they currently appear on the screen, click on the Current button.

   If you want the chromatogram(s) to be printed out with a layout different to that currently displayed on the screen, click on the **Define** button. This opens the **Chromatogram Layout** dialogue and enables changes to be made to the layout. Note that changes made here are only with respect to the report format and not the layout of the chromatograms as viewed on the screen.

Various report layout changes can be made in the **Chromatogram Layout** dialogue, for example:

- curves
- axes; you can select which y-axis that should be in the Y-Axis tab
- header information to be included on the same page of the chromatogram; select **Header** in the **Chromatogram Layout** dialogue and select the appropriate options (header information for imported chromatograms cannot be printed)

4. Click on **OK** when all changes in the **Chromatogram Layout** dialogue have been performed.

5. Click on **Print** or **Preview**.

9.7.1 Saving a report format

You can save a customised report format for later use. A saved report format also includes any selections you made in the **Chromatogram Layout** dialogue with respect to the report.

Note: The format cannot be saved with **Current** layout. The layout saved is always the one shown in **Define**.

All saved formats can be globally saved, i.e. made available for any user (if you have **Edit global lists** authorisation), or can be specific for the current user only. You can also save the layout as the default.

1. Select all the relevant information for your report format.

2. Click on the **Save as** button and type in a name for your format in the dialogue that is displayed.

3. Check the **Save as global format** or **Save as default report format** options as required. Click on **OK** to save the format.

9.7.2 Selecting a format

The newly saved format can be applied to any new report by selecting it from the list of **Report formats**.

Note: A report format saved with the **Current chromatogram** does not necessarily print the chromatogram as it appears on the screen. The layout (curve, axis etc.) that will be printed is the one which is found under **Define**. If you want to print the chromatogram as it appears on the screen, click on the **Current** (layout) button and then on **Print**.

Some global report formats are provided with the installation. Do not delete these formats, since you will then be unable to run the corresponding procedures.

If you want to print a number of results with the same report format, create a procedure to print one result and then perform a batch run for the required results (see Section 10.3.6 for details).

9.8 Run documentation

The full documentation of a run is stored within the result file. A few of these are described below. To open the **Documentation**, either select **View:Documentation** or click on the **View Documentation** toolbar button. To print documentation contents, click on the **Print** button in the **Documentation** dialogue (see Section 9.6.3). The contents can be saved as a new method by selecting **Method** page and clicking on **Save as**.

Variables

These are the parameters that were used during the run.

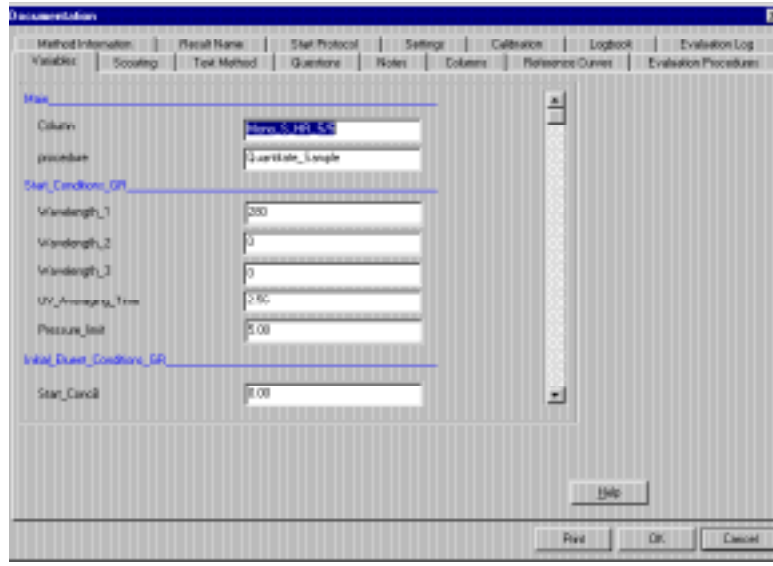


Figure 9-32. Documentation, Variables page.

Scouting

This displays the whole scouting scheme, with the values for the current result file displayed in yellow cells.

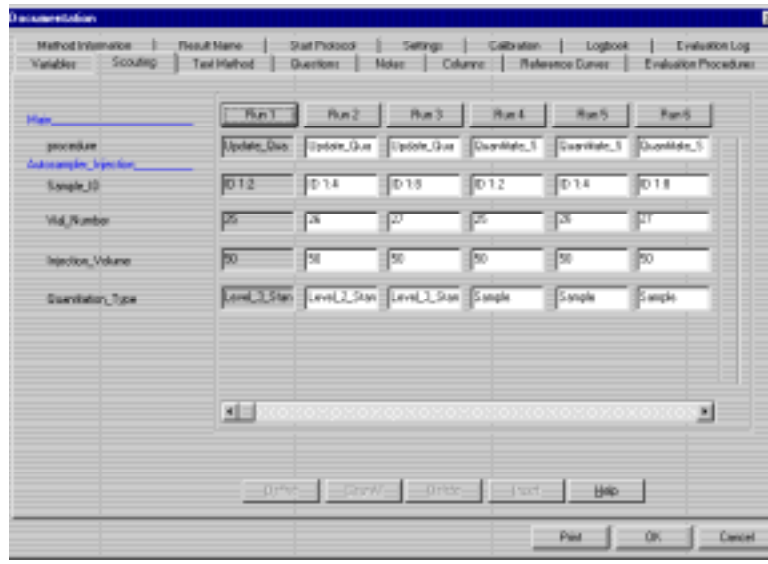


Figure 9-33. Documentation, Scouting page.

Notes

This displays notes that you have made at various points during the run. You are also able to enter new comments in the **Evaluation Notes** field.

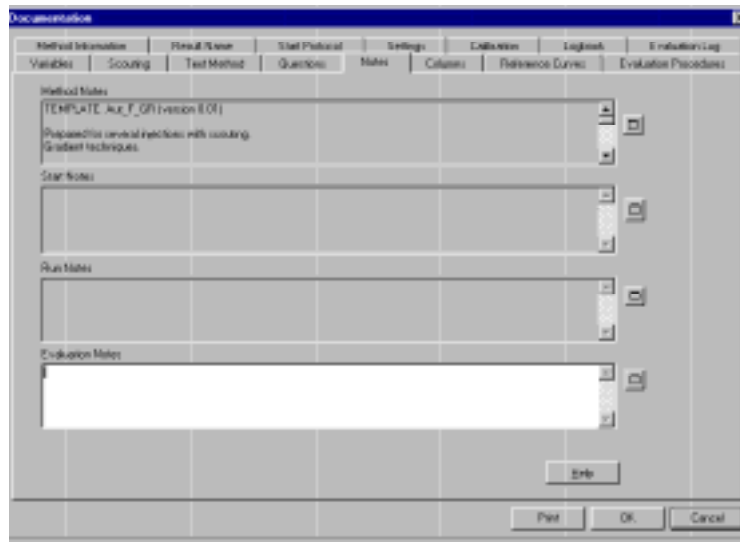


Figure 9-34. Documentation, Method Notes page.

Calibration

This displays what system calibrations were made, when and by whom.

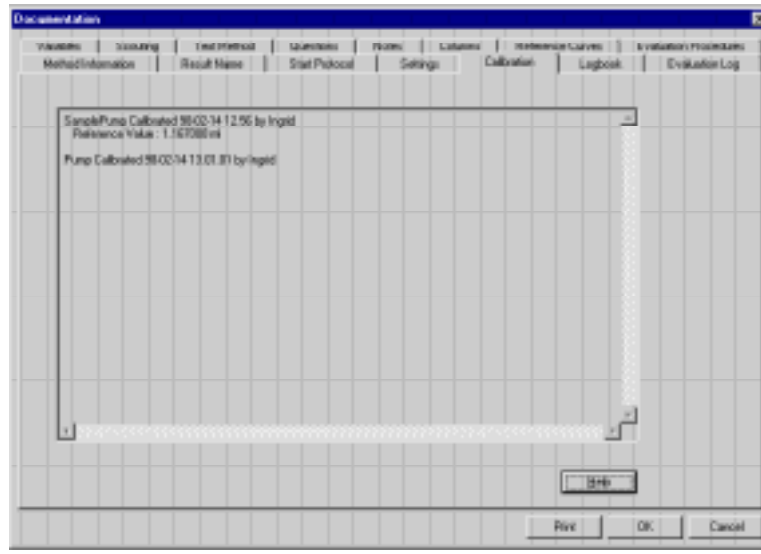


Figure 9-35. Documentation, Calibration page.

Logbook

This displays what exactly happened during a run. It is possible to view information concerning alarms, the method, manual changes during the run, errors and the system.

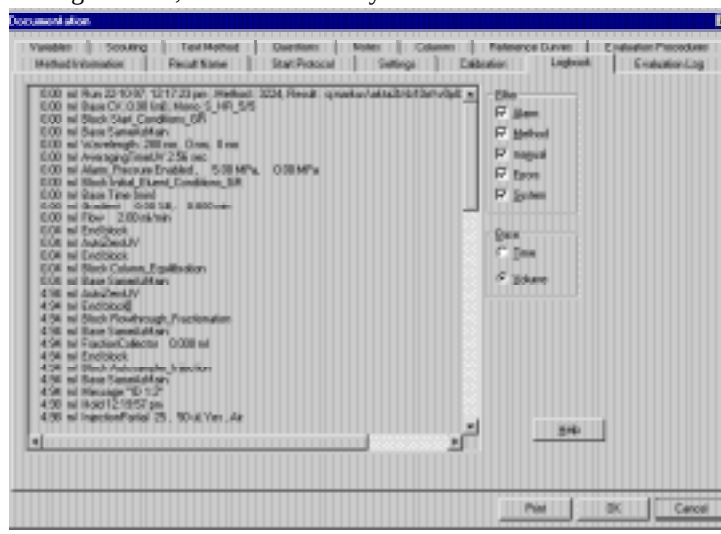


Figure 9-36. Documentation, Logbook page.

Evaluation Log

This lists all of the evaluation operations that you have performed for the current result file for all sessions, including procedures executed at the end of the method.

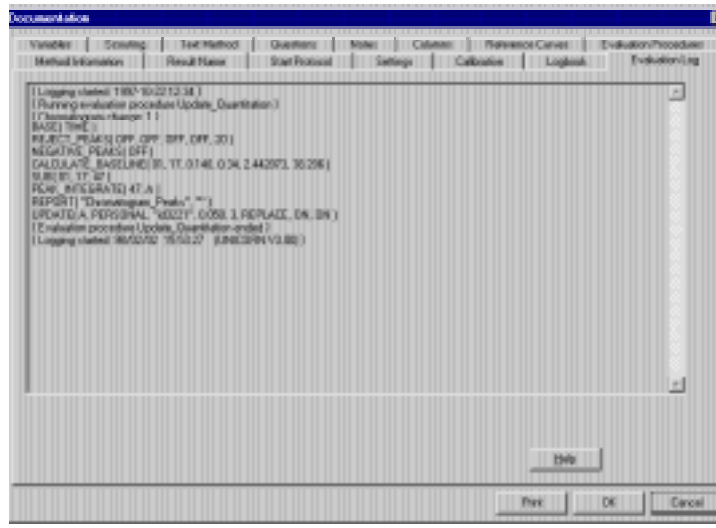


Figure 9-37. Documentation, Evaluation Log page.

9.9 Exiting Evaluation

If you want to quit from the Evaluation module of UNICORN, select **File:Exit**. You will then be asked if you want to save the results of the evaluation session that you have performed. If you answer **Yes**, the previous version of that result file will be unconditionally overwritten. This may be undesirable if you have included the current result file within an evaluation procedure batch run (see Section 10.3.5).

10 Evaluating results

This chapter will mainly describe how to:

- integrate peaks
- automate evaluation operations
- export data and curves

10.1 Integrating peaks

UNICORN allows you to identify and measure on a curve peak areas, retention times, peak widths etc., using peak integration.

10.1.1 Baseline calculation for integration

Integrating peaks is divided into two steps: calculating the baseline and calculating peak areas. As a correct baseline is crucial for accurate calculation of peak areas, several ways of calculating the baseline are available in UNICORN:

- Using the **Calculate baseline** instruction for automatic calculation of the baseline, which gives, in most cases, a very accurate measurement. This is the most common alternative and it is strongly recommended that you read the information contained within Appendix D.2 which describes the principles of baseline creation.
- A blank run curve with the same chromatographic conditions as corresponding with the sample can be used as the baseline for peak integration. Another approach that may improve the peak integration if a blank run is available is to first subtract the blank run from the source curve (see Section 9.3.3) and then perform peak integration on the resulting curve using the **Calculate baseline** option.

  In addition to blank runs, it is possible to select any curve present in the current chromatogram, e.g. an edited baseline (see Section 10.1.5), as baseline.

- Using a **Zero baseline**, i.e. no baseline subtraction at all.
- Reusing an already existing baseline for the selected curve by selecting the **Correlated baseline** option. This is the default alternative whenever possible.

10.1.2 Performing a basic integration

To perform a basic integration:

1. Select **Integrate:Peak integrate** or click on the **Peak Integrate** toolbar button. The **Integrate** dialogue is displayed.

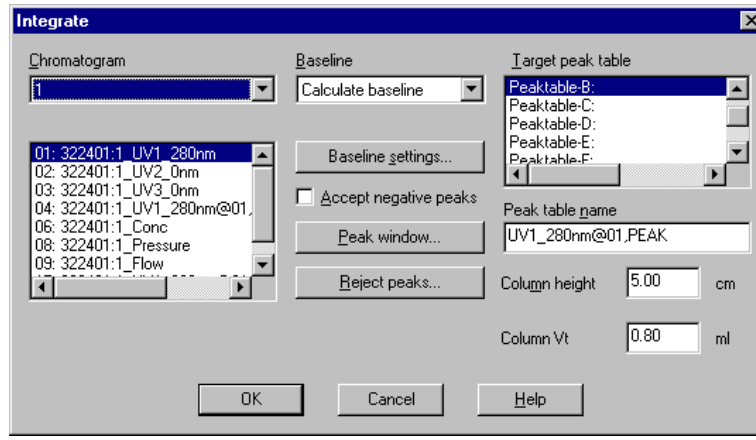


Figure 10-1. Integrate dialogue.

2. Choose the source curve to be analysed, usually the first UV curve, and a peak table destination from the peak table list. Any chromatogram can contain up to eight peak tables, designated A-H.

3. Make the appropriate **Baseline** selection from the above-mentioned possibilities. The **Calculate baseline** option with the default settings for the parameters is the most common choice.

4. Click on **OK** to perform the peak integration when you are satisfied with your selections.

Following integration, the peaks in the chromatogram will be automatically labelled with their respective retention times. The start and end point of each peak will be marked by drop-lines.

The peak table will be displayed underneath the active chromatogram.

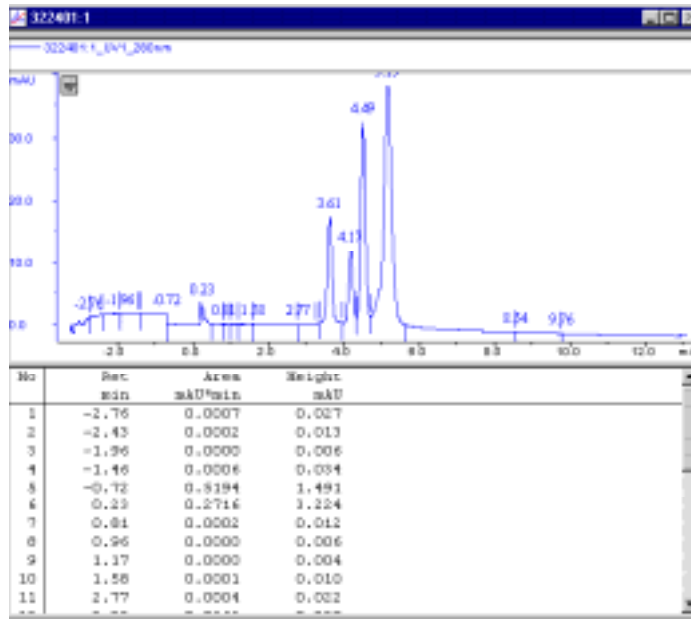


Figure 10-2. The results after peak integration.

In addition to peak areas, several other peak characteristics such as retention time and peak width are automatically calculated. The characteristics displayed in the peak table may be selected in the **Chromatogram Layout** dialogue, **Peak Tables** tab (see Appendix D.3).

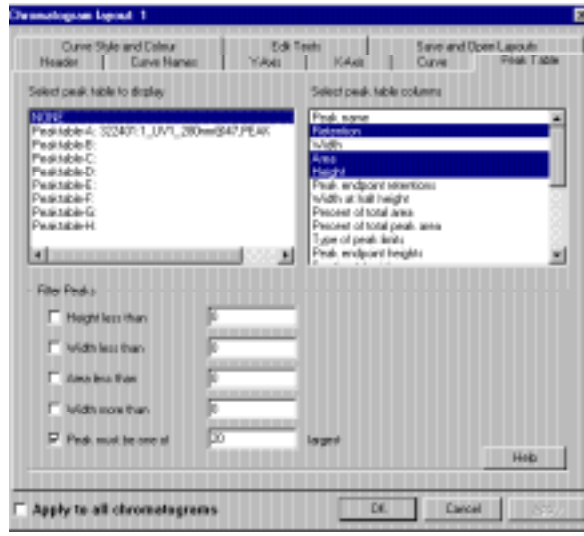


Figure 10-3. Chromatogram Layout dialogue, Peak Table tab.

Select the options that you want to be displayed from the **Select peak table columns list**. Most characteristics are automatically calculated for each integrated peak when the peak integrate function is used, although only the selected items will be displayed in the peak table.

Changing peak labels

As alternatives to using retention times as peak labels, the peaks can be sequentially numbered or be marked with specific identification tags. The choice of label type is made in the **Curve Style and Colour** tab in the **Chromatogram Layout** dialogue (see Section 9.2.5). Note that the labels may be displayed vertically for each peak by deselecting the **Horizontal text** option. If you do not want to view the peak labels, e.g. for presentation purposes, copy the curve into the temporary chromatogram (see Section 9.1.2)

Filtering peaks from view

It is possible to temporarily remove peaks from display in a peak table based upon the criteria you determine.

1. In the **Chromatogram Layout** dialogue, click on the **Peak Tables** tab.

2. Select (check) the filter criteria in the peak table and specify the values used to filter the peaks, i.e.the minimum height, width and area, the maximum width as well as a specified number of the largest size peaks. Click on **OK**.

If you later want to include the peaks again you have to deselect the options. The difference between **Filter peaks** and **Reject peaks** is that the latter function permanently excludes peaks from the integration and affects the calculation of total peak area etc.

10.1.3 Optimising peak integration

If the results from the peak integration are unsatisfactory, there are several possibilities to improve the results.

1. Changing the parameters used in the **Calculate baseline** instruction can help to optimise the integration result (see Section 10.1.4). The baseline parameters can be altered in two possible locations (i) in the **Calculate Baseline** dialogue displayed after selection of **Integrate:Calculate baseline**, or; (ii) by selection of the **Baseline settings** command button in the resulting **Integrate** dialogue. These dialogues function identically. However, the baseline is immediately used in an peak integration.

2. It is possible to exclude peaks from integration based upon criteria you determine. Click on the **Reject peaks** command button in the **Integrate** dialogue. In the dialogue that is displayed, select (check) the criteria and parameters by which peaks will be excluded from the integration. You are able to define the minimum height, width and area, the maximum width as well as a specified number of the largest size peaks. The default criteria is to include only the 20 largest peaks.

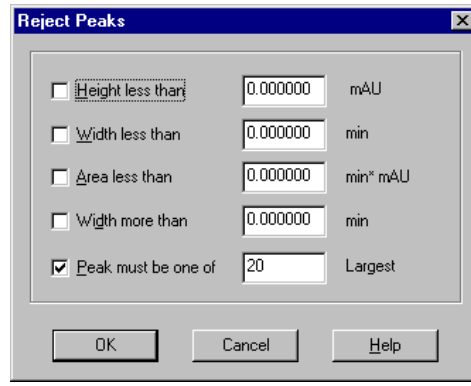


Figure 10-4. Reject Peaks dialogue.

3. To select only a part of a curve for integration, click on the **Peak window** button in the **Integrate** dialogue. A chromatogram window will open containing the curve and two vertical cursor lines. These lines can be dragged to define a region between them that will be analysed. Alternatively, x-axis values for the **Left limit** and **Right limit** may be typed in. Click on **OK** to return to the main dialogue. The baseline will be calculated from the whole curve, but calculation of the areas beneath the peaks is only performed on the selected section of the curve. The default peak window includes the entire curve.

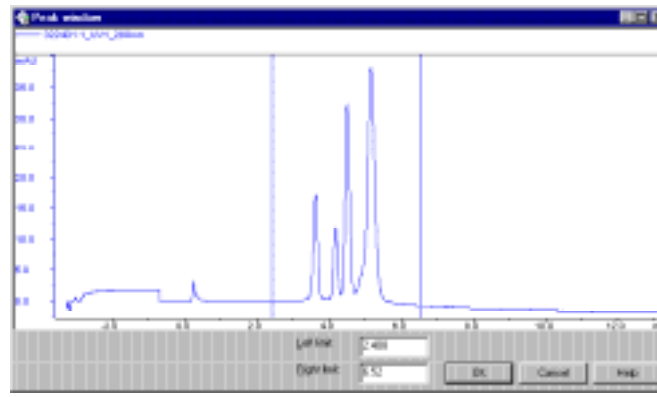


Figure 10-5. Peak Window chromatogram window.

4. If you want to include negative peaks in the integration, select (check) **Accept negative peaks** in the **Integrate** dialogue (or in the **Calculate baseline** dialogue). The negative peaks will be reported as negative areas in the peak table. By default, negative peaks are not included in the integration.

5. In the event that the automatic baseline calculation does not produce a satisfactory baseline, it is possible to edit the baseline manually by inserting and deleting baseline points. This is done with the **Integrate:Edit baseline** function (see Section 10.1.5).

6. The **Integrate:Edit peak table** function (see Section 10.1.6) allows you to manually adjust the peak start and end points, split and join peaks.

10.1.4 Optimising the baseline parameters

The first choice when trying to optimise the peak integration is to change the baseline parameters. A brief description of the parameters and a suggested way of estimating settings for the baseline parameters from the source curve, is given in Appendix D.2. The **Integrate:Edit baseline** function may provide additional information about the cause of the unsatisfactory peak integration by revealing where the baseline points have been placed by the automatic baseline calculation algorithm.

When optimising the baseline calculation, a change in the **Slope limit** will very often give the expected result. An example of the effect of a too high **Slope limit** is that the up-slopes of the peaks may be recognised as baseline segments. Baselines that deviate substantially from the source curve may arise from a combination of a too long **Shortest baseline segment** value with a too high **Slope limit**.

A recommended strategy is to change the baseline parameters step by step and to check the resulting baseline after each change. The size of the initial change depends on the cause of the peak integration problem, but some general guidelines can be given. When the desired effect is achieved it is advisable to go back and check a parameter value in between the two last settings to avoid an unnecessarily low or high value. The default baseline parameters can be restored by clicking on the **Default** command button.

Baseline parameter	*Recommended initial change*
Shortest baseline segment	20-50%
Noise window	10-30%
Slope limit	25-50%
Max baseline level	Usually not necessary to adjust

Below are some examples of common problems with peak integration and baseline calculation and suggestions for improvements. The following problems will be discussed:

- Baseline does not follow the source curve
- Peak limits too high up on the peaks
- Noise detected as peaks
- Peaks missing
- Baseline on top of peaks

Baseline slope does not follow the source curve

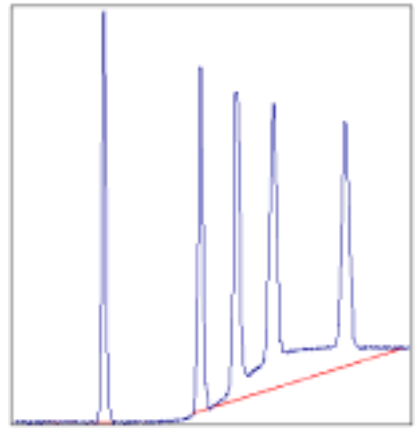


Figure 10-6. Baseline calculation with default parameters.

The calculated baseline does not follow the source curve, because short curve segments between peaks in the middle of the chromatogram are not identified as baseline segments. If you decrease the **Shortest baseline segment** by 50% the following baseline is calculated.

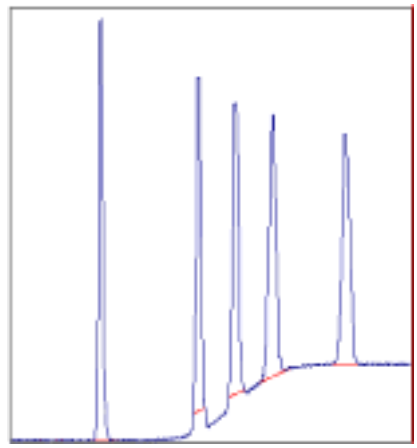


Figure 10-7. Baseline calculation with 50% lower Shortest baseline segment.

The baseline is however still unsatisfactory, due to the high slope of the short segments in the region between the second and fourth peak which are still not identified as baseline segments. If you increase the **Slope limit** by a factor 2.5, a correct baseline is calculated.

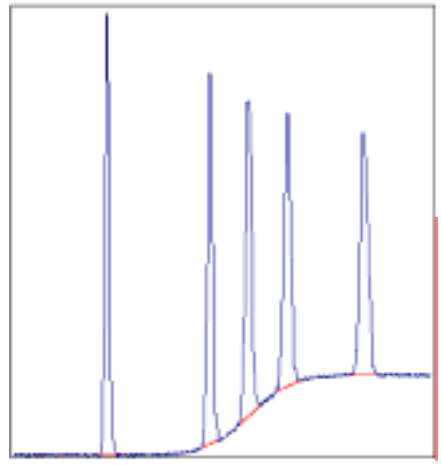


Figure 10-8. Correct baseline parameters.

Peak limits too high up on the peaks

This peak integration problem is in most cases caused by a too high value for the **Slope limit** and/or a too high value for the **Noise window**. This can be encountered when the chromatogram includes a very large flow-through or solvent peak. The large peak affects the calculation of the default baseline parameters, leading to too high values for **Slope limit** and **Noise window**.

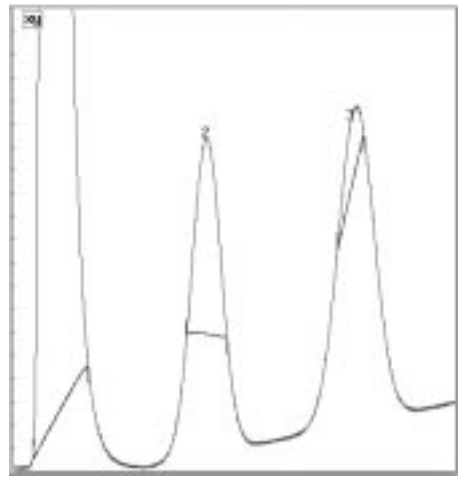


Figure 10-9. Peak start and end points on peaks caused by too high Slope limit.

Note the difference between the situations in Figures 10-6 and 10-8. In Figure 10-6, no baseline segments were detected between the second and fourth peaks (there are no blue crosses is this region when **Edit baseline** is selected). The baseline follows the curve as a best fit, and cannot be drawn above the curve unless **Accept negative peaks** is selected. In Figure 10-8, baseline segments are detected on the up and down slopes of the peaks (marked by blue crosses in these regions when **Edit baseline** is selected).

By decreasing the **Slope limit** in Figure 10-8 considerably, a better baseline can be constructed, leading to an improved peak integration.

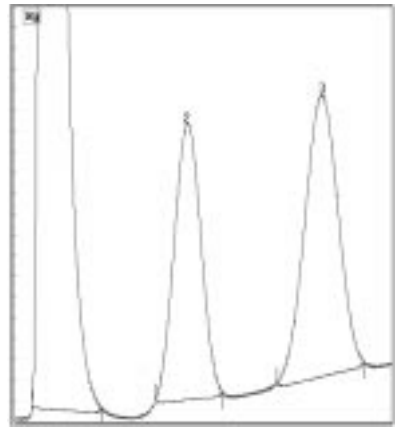


Figure 10-10. Peak integration with decreased Slope limit.

You may also have to decrease the **Noise window** to get a perfect peak integration.

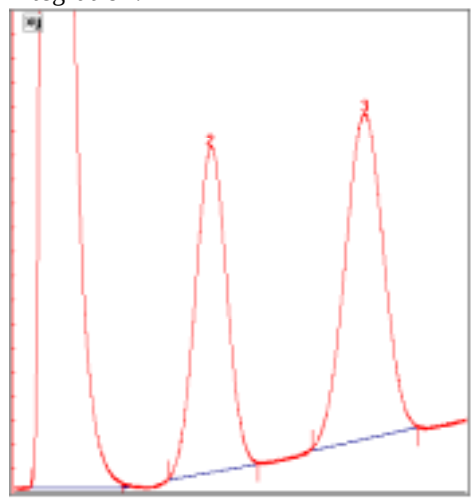


Figure 10-11. Correct baseline after decreasing both Slope limit and Noise window.

An alternative approach is to exclude the large peak from the peak integration, as its presence affects the default baseline parameters and the retention and area of the large peak is in most cases not interesting. Using the **Operations:Cut curve** function, the appropriate region of the chromatogram can be selected. The peak integration can then in most cases be performed with default baseline parameters on the cut curve. You can not use the **Peak window** function to remove the large peak as the baseline is calculated for the entire curve.

Noise detected as peaks

Sometimes you get too many peaks after the peak integration, usually due to that noise on the baseline is erroneously detected as peaks.

The cause of this problem is that the **Noise window** parameter is too low. Increase the **Noise window** parameter in the baseline calculation. This may in some cases result in peak limits too high up on the peak slopes, see example above.

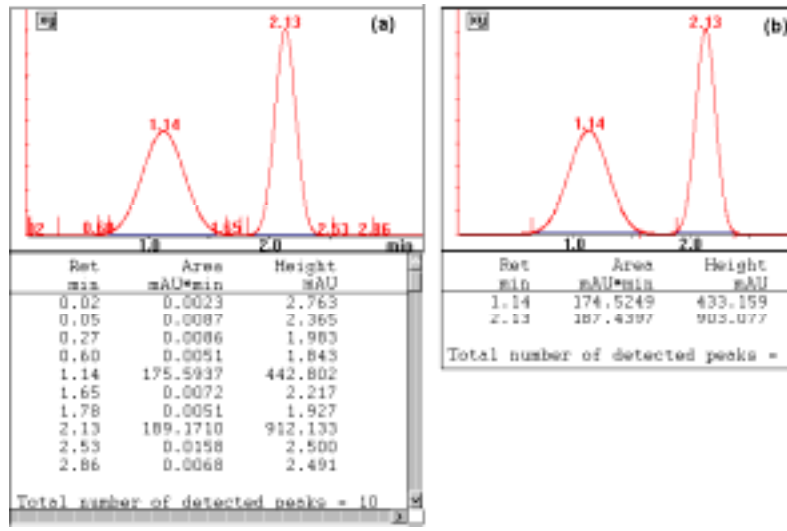


Figure 10-12. (a) Noise detected as peaks; (b) Peak integration after increase of Noise Window.

Another possibility is to use the **Reject peaks** function in the **Integrate** dialogue to reduce the number of peaks based on an appropriate criterion, e.g. the number of peaks or the minimum peak height.

Peaks missing

In case obvious peaks are not detected in the peak integration, a probable cause is that the **Noise window** parameter is too high.

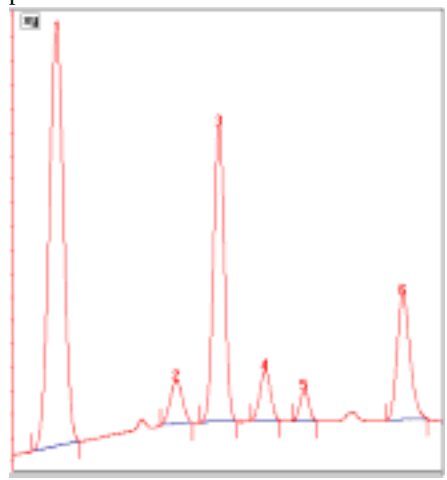


Figure 10-13. Peak integration with too high Noise window.

Decrease the **Noise window** parameter until the peaks are detected.

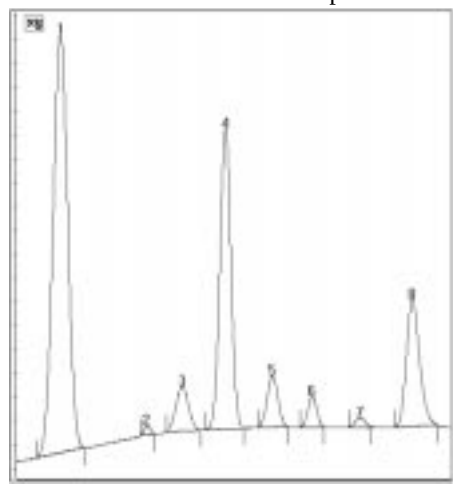


Figure 10-14. Correct peak integration after decreasing Noise window.

Another possible cause of missing peaks is that an improper reject criterion has been used. Check the criteria used for **Reject peaks** in the **Integrate** dialogue as well as Filter peaks in the **Chromatogram layout** function.

Baseline on top of peaks

In rare cases the top of a broad flat peak will be incorporated as a baseline segment.

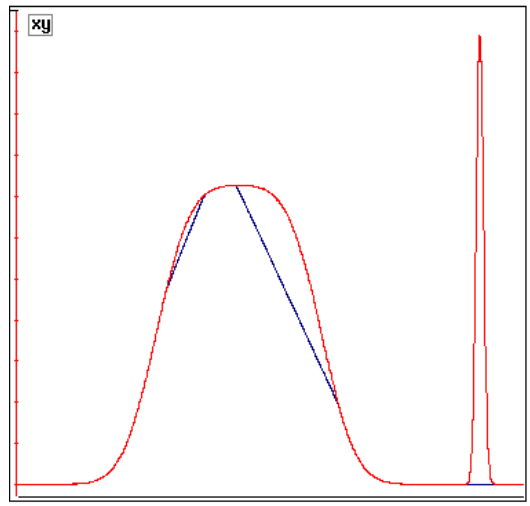


Figure 10-15. Baseline segment on top of peak.

This is one of the very few situations where it is useful to change the **Max baseline level**. Measure the height of the flat plateau of the peak using the **XY** icon on the chromatogram (see Section 10.1.7). Insert a value somewhat lower than the plateau height as the **Max baseline level** in the baseline calculation.

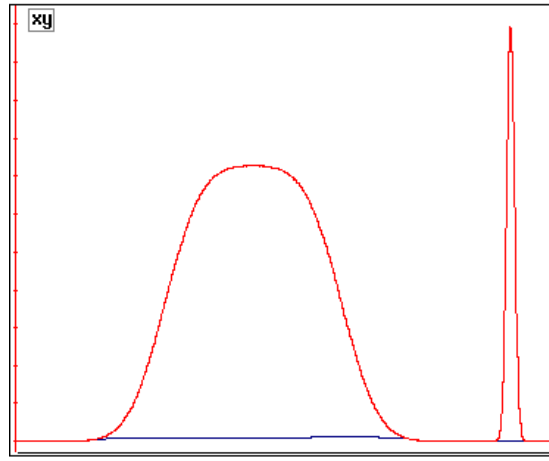


Figure 10-16. Correct baseline after decreasing Max baseline level.

If there are several rather short segments that erroneously have been incorporated in the baseline, an alternative remedy is to increase the **Shortest baseline segment** setting.

10.1.5 Manually editing a baseline

Once a baseline has been calculated, it is possible to add or remove baseline points on it and then draw a new baseline from the new set of data points. The edited baseline curve can then be used in a new peak integration.

1. Select **Integrate: Edit baseline**.

2. Choose the desired baseline from the displayed dialogue and click on **OK**. A window will appear displaying the baseline and the curve from which it was calculated. Additionally, blue crosses are displayed (the baseline points) and their co-ordinates in the **Point list**.

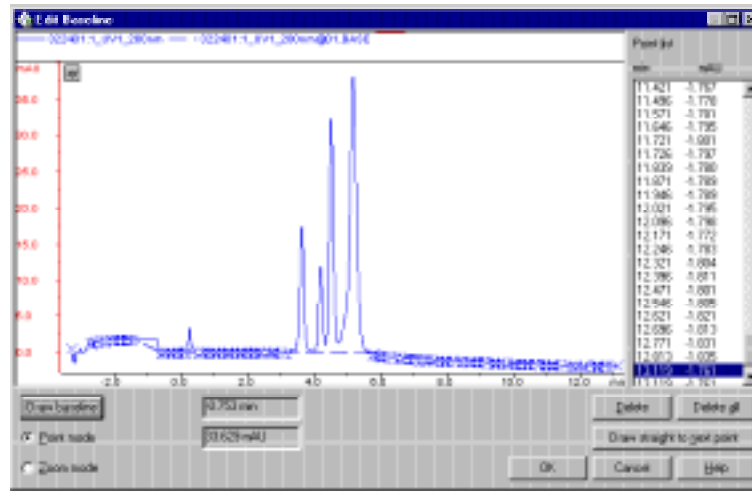


Figure 10-17. Edit Baseline chromatogram window.

Insertion of baseline data points

Select the **Point mode** button. Click on the left mouse button to place a new baseline point on the chromatogram. Each new point is represented by a cross and its co-ordinates are automatically entered into the **Point list**. This is useful when, for example, you want the baseline to go up to a high valley between two peaks.

To make your task easier you can click on **Zoom mode** and zoom in on specific regions of the chromatogram and then insert baseline data points. The right mouse button menu allows you to undo the last zoom step with **Undo zoom** or to reset the default zoom scale with **Reset zoom**.

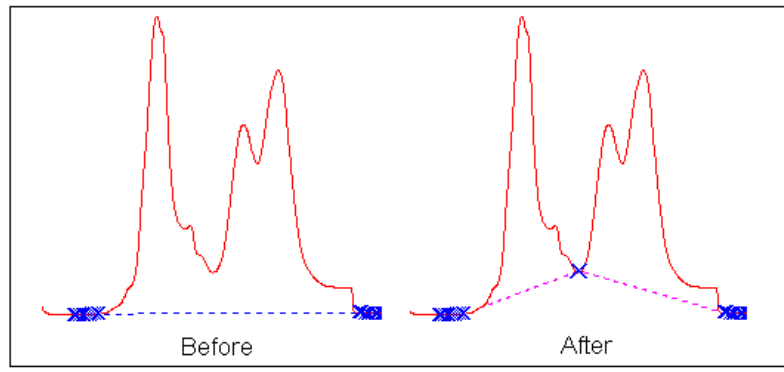


Figure 10-18. Baseline before and after editing.

Deleting baseline data points

If you want to delete a data point from the **Point lis**t, select the appropriate point in the list and press the **Delete** button. Alternatively, double click on the unwanted data point entry in the list to delete it. **Delete all** removes all baseline data points.

Drawing the new baseline

When you are satisfied with your baseline point selection, click on the **Draw baseline** button. The new baseline curve will be drawn as a spline function based on the previous and the new points. The spline function is guided by the points, but does not necessarily pass through them. You may also force a straight baseline between two points by selecting the first of the two points in the point list and then clicking on the **Draw straight to next point** button.

Click on **OK**, and the new baseline will be saved with the default name **Edited Baseline**. This may now be used as the baseline in a new peak integration.

10.1.6 Adjusting the peak limits

Once a peak table has been generated using the appropriate baseline, it is now possible to split or join peaks and to manually adjust the peak start and end points. The peaks will then be renumbered and the peak areas will be recalculated.

1. It is recommended that you first access **Curve Style and Colour** tab in the **Chromatogram Layout** dialogue, and select either **Number** or **Retention** for labelling the peaks. The former option will sequentially number each of the peaks in the chromatogram which is opened during the edit mode of a peak table. The latter option will display the retention volume or time for each peak.

2. Select **Integrate:Edit peak table**. Select the desired peak table from the displayed dialogue. Note that name of the baseline on which the selected peak table was based, is displayed at the bottom of the panel.

3. Double click on the desired peak table in the list or click on **OK**. A chromatogram window is displayed containing the selected peak table with corresponding curve and baseline. The various editing features are described below. As an aid, it is possible to use the zoom function.

4. Once you have completed your changes, click on **OK** and verify the destination of the new (edited) peak table.

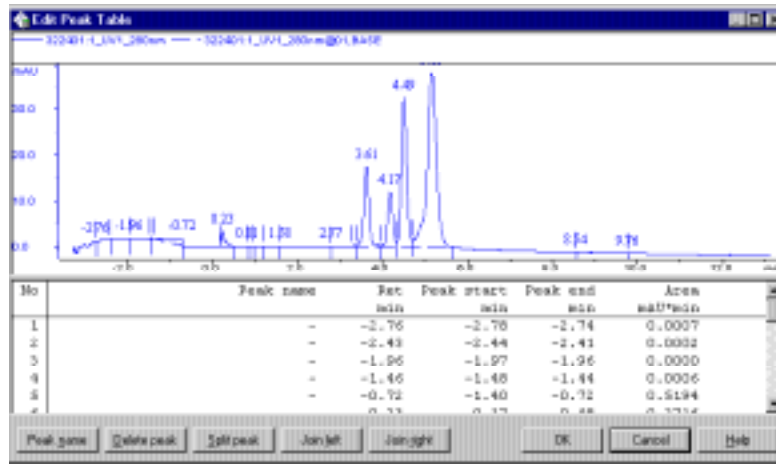


Figure 10-19. Edit Peak Table chromatogram window.

Deleting a peak in the peak table

To delete a peak from the table, click on the peak in the chromatogram or in the peak table and click on the **Delete peak** command button. Note that the remaining peaks will be renumbered after the deletion.

Splitting a peak

A peak is defined within delimiting drop-lines to the left and right of the peak. It is possible to split the peak into two new peaks by inserting a drop-line. The drop-line is always inserted at the middle point between two existing drop-lines. The area under each new peak will not be the same if the symmetry of the original peak was not perfect.

To make a split, select the desired peak in the list or mark in the curve and press the **Split Peak** button.

Note: The peaks will be renumbered according to the split. Refer to the description below about adjustment of the drop-lines.

Joining a peak

It is possible to join the areas of adjacent peaks if separated by a drop-line.

1. Select a peak either on the chromatogram or in the peak table.

2. Click on **Join left** or **Join right** if you want the peak to be joined with the peak to its left or right respectively. The original intervening drop-line is removed and all peaks are renumbered.

Adjusting peak start and end points

The beginning of each peak is marked with a drop-line above the curve, and the end of each peak is marked with a drop-line below the curve.

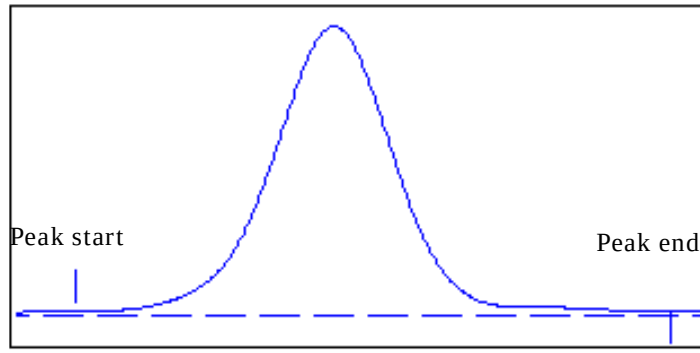


Figure 10-20. A drop-line at the start and end of a peak.

Where there are two peaks beside one another, the end of the first peak will be at the same point as the beginning of the next peak. Thus, there will be a drop-line below and above the line at the same point.

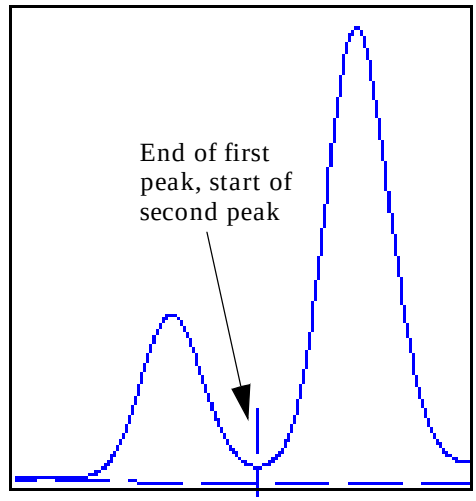


Figure 10-21. A drop-line between two peaks.

It is possible to move the drop-lines for a selected peak and thus affect the area beneath the peak.

1. Click on the peak of interest on the curve or in the peak table and two vertical cursor bars become superimposed on the left and right drop-lines that delimit the selected peak.

2. Drag the left and right drop-line bars to define the new left and right limits respectively for the selected peak. The drop-lines can never be moved beyond any other drop-line. The new left and right limits are now represented by a drop-line above and below the curve respectively, and the peak areas are automatically recalculated.

3. Drop-lines on preceding or following peaks can be similarly adjusted. Movement of these drop-lines can be up to, but never beyond, any other drop-line. A drop-line may also not be moved beyond a point where the peak meets the baseline.

Identification names for peaks

Double click on the peak of interest in the list. A dialogue is displayed with the peak number, its retention value and a text field in which you can enter a specific tag for that peak. Click on **OK**. You will see the peak names in the chromatogram only if you have earlier accessed **Curve Style and Colour** tab in the **Chromatogram Layout** dialogue, and selected **Names** for labelling the peaks.

10.1.7 Measuring retention time and peak heights

It is possible to determine the co-ordinates of any point on a curve and thus obtain values for retention and peak height. This is a useful tool for many other functions, such as for measuring the parameters used in baseline calculations. Co-ordinates can be obtained in two ways:

- Direct measurement
- Viewing peak table data

Direct measurement

1. Double click on the small **XY** icon within the chromatogram area at the top of the y-axis. The active **XY** box contains coloured text which shows the x-axis co-ordinate and y-axis co-ordinate, both with their respective units. The colour and units of the y-axis information corresponds to a specific curve of the same colour.

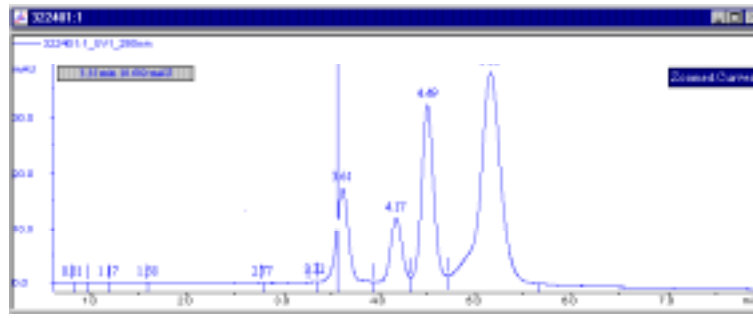


Figure 10-22. Active bar linked to XY box for determining curve point co-ordinates.

2. Click on the desired curve legend and the correct y-axis is displayed.

3. A vertical cursor line can also be seen on the chromatogram which is the same colour as the selected y-axis scale. Use the mouse pointer to drag the line back and forth along the chromatogram. Co-ordinates, corresponding to retention time and peak height, will change accordingly in the active **XY** box.

4. Double click on the active **XY** box to close this function.

Viewing peak table data

The retention time and amplitude of any peak can be directly viewed in a peak table after an integration, if selected for in the **Chromatogram Layout** dialogue.

10.1.8 Measuring HETP

HETP (height equivalent to a theoretical plate) calculations allow you to check how well the column has been packed.

1. Perform a run with injection of a non-interactive substance, for example, a small volume of acetone. Note that the injection must be at zero time.

2. In the **Integrate** dialogue, type in the column height (cm) in the appropriate field area. Perform the peak integration according to your other selected parameters.

3. To view the results of the integration, select **Plate height (HETP)** in the list within the **Peak Table** tab of the **Chromatogram Layout** dialogue.

Every peak will have a HETP value. A narrow peak gives a low value corresponding to a well packed column. A broad peak gives a high value, indicating a column that is not optimally packed.

HETP is calculated as follows:

HETP = L/N

$N = 5.54 * (V_R/w_h)^2$ assuming a Gaussian peak

where

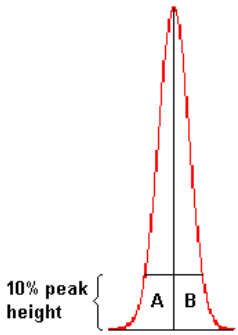
N = no. of theoretical plates

L = bed height in cm

V_R = peak retention (elution) volume or time

w_h = peak width at half height expressed in the same units as VR

10.1.9 Measuring peak asymmetry



This function can be used in combination with HETP to help assess column performance. A perfect peak will have no asymmetry and, after peak integration, give a value of 1.0. Any value less than 1.0 means that there is a left skew, i.e. the asymmetry falls on the leading side (left) of the peak. The reverse is true for values greater than 1.0 where the asymmetry comes on the tailing side (right) of the peak.

To view the asymmetry data select **Asymmetry** in the list within the **Peak Table** tab of the **Chromatogram Layout** dialogue. Click on **OK** and return to the chromatogram window.

Asymmetry = width B / width A, where A and B are the partial peak widths measured at 10% of the peak height, with A representing the leading part of the peak and B the tailing part of the peak.

10.1.10 Measuring resolution

Resolution is a measure of the relative separation between two peaks and can be used to determine if further optimisation of the chromatographic procedure is necessary. If the resolution value is 1.0, then 98% purity has been achieved at 98% of peak recovery, provided the peaks are Gaussian and approximately equal in size. Baseline resolution requires that the resolution value is greater than or equal to 1.5. At this resolution, purity of the peak is 100%.

To view the resolution data, select **Resolution** in the list within the **Peak Table** tab of the **Chromatogram Layout** dialogue. Click on **OK** and return to the chromatogram window. The resolution value for each peak shows the resolution with respect to the previous peak.

$$R = \frac{(V_{R2} - V_{R1}) \times 1.177}{w_{h1} + w_{h2}}$$

where:

$V_{R2} > V_{R1}$

V_{R1} = retention (elution) volume for peak 1

V_{R2} = retention (elution) volume for peak 2

w_{h1} = peak width at half height for peak 1 (for Gaussian peaks)

w_{h2} = peak width at half height for peak 2 (for Gaussian peaks)

10.2 Other evaluations

10.2.1 Peak purity and peak identification

Ratios between UV curves measured at different wavelengths give useful information about peak purity or peak identity. The **Operations:Divide** function can be used when you have a result file with run detected at more than one wavelength.

1. Before dividing the curves, you must make sure that both curves have a baseline close to zero AU. This can be achieved with baseline subtraction.

2. Create a baseline for each UV curve according to the procedures detailed in Sections 10.1.1 and 10.1.2. Subtract the baselines from their respective UV curves using **Operations:Subtract**.

   Alternatively, you can subtract the corresponding blank runs from the UV curves if such exist.

3. When you have achieved two curves with a zero baseline, select **Operations:Divide** and select the two curves for division. You have the option to set **Threshold** values by checking the option and setting the threshold value for each curve. This sets the quotient to 1.0 if either of the sample values is closer to zero than the threshold value. This is to prevent very high quotient values being created if division is performed with values close to zero. Very low quotient values are also prevented. **Threshold** values are suggested by UNICORN although these can be changed.

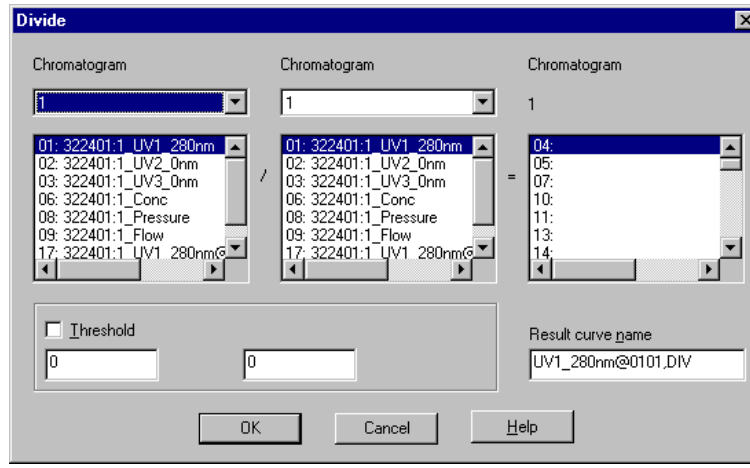


Figure 10-23. Divide dialogue.

4. The resulting curve can then be filtered by **Operations:Smooth** (see Section 9.3.2). It is suggested that you smooth using the median filter to remove noise that appears as spikes or occurs in a small area of the curve.

The ratio can be used to check peak purity. If the peak is pure, the absorbance spectra are the same over the whole peak and therefore the ratios should remain constant. If the absorbance ratio is not the same over the whole peak, then the peak is probably not pure.

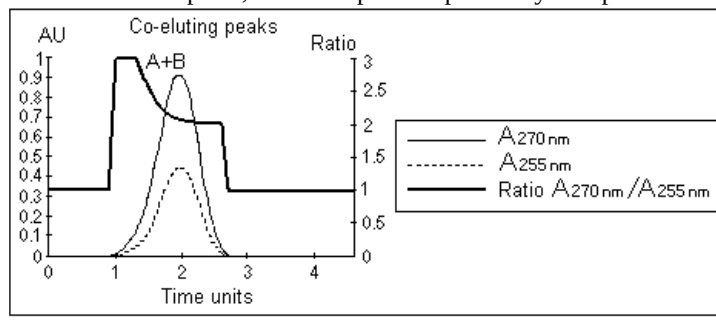


Figure 10-24. Simulated chromatogram of two co-eluting components with differing absorbance spectra and a small difference in retention time

The resulting ratio can also be used for peak identification as different compounds have a specific ratio between absorbencies at different wavelengths.

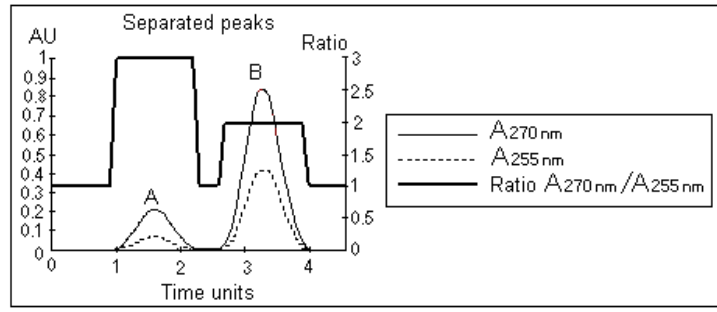


Figure 10-25. Simulated chromatogram of two components with differences in their absorbance spectra.

10.2.2 Finding the slope values for Peak Fractionation or Watch instructions

With ÄKTAdesign systems it is possible to only collect peaks during fractionation. Peak fractionation parameters are set in the Method editor with the instruction Peak_FracParameters. StartSlope and EndSlope values are set. Described below is how to find suitable slope values for a particular run.

It is also possible to set up conditional (Watch) instructions which allow the progress of a run to be determined by the events during the run, e.g. start collecting fractions when the first peak emerges. The slope of the curve may be set as a condition used to satisfy a Watch condition in the method during the run. It is therefore important to use accurate slope values for the specific Watch instruction parameter.

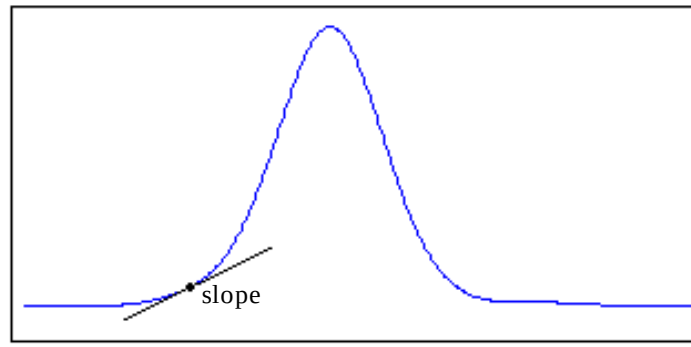


Figure 10-26. The slope of a curve.

To determine slope values, you must first make a run with the sample you intend to purify. Then use this result to find slope values in the Evaluation module:

1. Ensure that you have selected **Time** as the x-axis scale for retention in the **Chromatogram Layout** dialogue, **X-Axis** tab.

2. Select **Operations:Differentiate**. Select the desired (UV) curve, check that a **First order** calculation is selected and click on **OK**. The differentiated curve will appear in the active chromatogram.

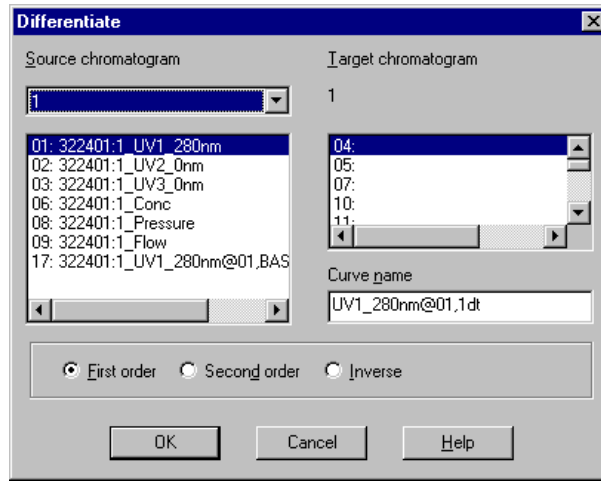


Figure 10-27. Differentiate dialogue.

3. Measure the y-axis values on the differentiated curve by clicking on the **XY** icon, choosing the y-axis differentiate scale and reading the curve co-ordinates in the active **XY** box. It may be necessary to smooth the differentiated curve. The units for the differentiated curve is mAU/min or AU/min. Co-ordinates are based upon the position of the vertical line on the chromatogram in relation to where it bisects the curve. Any y-axis value for the differentiated curve is the UV curve slope at the selected retention point.

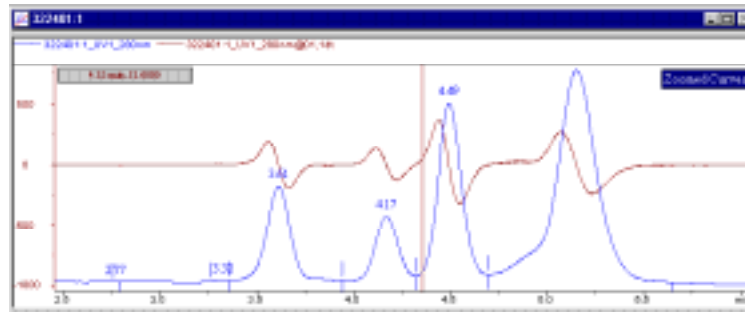


Figure 10-28. Measurement of the Slope limit after differentiation.

4. Use the zoom function to magnify the curve over an appropriate area. Place the vertical cursor bar at the beginning of a peak where you want the Watch conditions to be fulfilled, i.e. where the slope becomes higher. Read the actual slope value in the active **XY** box.

   When using the PeakFractionation instruction for ÄKTAdesign systems, measure the slope at the beginning and end of the smallest flattest peak to collect and use these values.

5. In the Method editor, enter the slope value as a parameter for the Watch instruction or enter the StartSlope and EndSlope values in the Peak_FracParameters instruction.

10.2.3 Creating a curve

It is possible to create a curve based on any external measurements.

1. Select **Operations:Create Curve**. In the dialogue that is displayed, select one or several help curve(s).

2. Select the minimum and maximum values of the y-axis. Also choose the appropriate units from the list that is displayed when you click on the drop-down arrow. The help curve determines the min and max values for the x-axis.

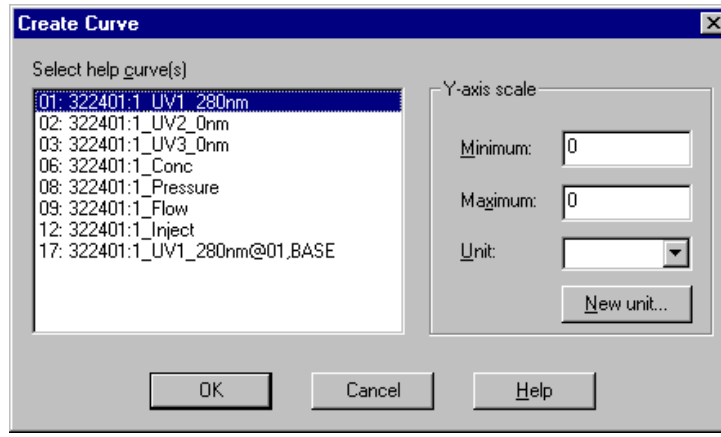


Figure 10-29. Create Curve dialogue.

3. If you want to create new unit, click on the **New unit** button and enter the new unit name and number of decimal places the values will receive.

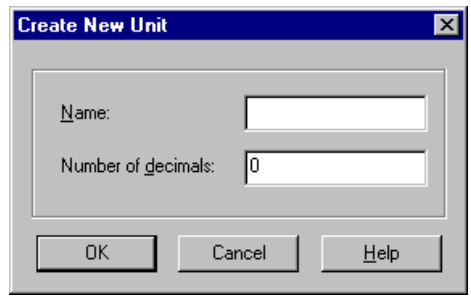


Figure 10-30. Create new unit dialogue.

Click on **OK** to return to the **Create curve** dialogue and again on **OK** when you have made your selections there.

4. With **Point Mode** selected, you can use the left button to insert new curve points on the chromatogram. The co-ordinates of each new point are automatically entered into the **Point list**.

   The co-ordinates of the mouse cursor are displayed beneath the curves thus allowing you to precisely position a new data point. The co-ordinates for the new curve are always displayed. Selecting **Zoom mode** allows you to use the mouse to select an area of the view and zoom in. The right mouse button menu allows you to undo the last zoom step with **Undo zoom** or to reset the default zoom scale with **Reset zoom**. In **Zoom mode** you can also drag the vertical cursor line and the co-ordinates presented reflect where the line bisects the curve of the same colour.

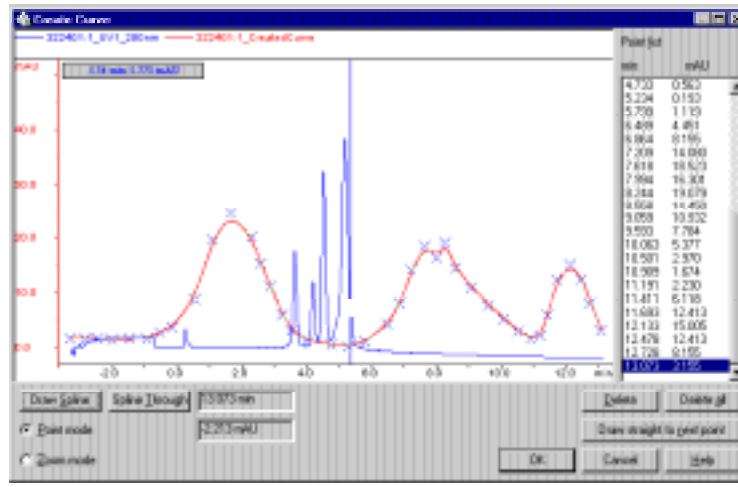


Figure 10-31. Create Curve chromatogram window.

5. To delete a point from the **Point list**, double click on the appropriate choice in the list. Alternatively, select the co-ordinates in the list and click on the **Delete** button. To delete all of the points in the list, click on the **Delete All** button.

6. To draw the curve, click on either **Draw spline** or **Spline through**. **Draw spline** creates a smooth curve from the data but does not necessarily pass through every point that you have entered. By contrast, **Spline through** creates a curve that passes through all of the data points.

7. In cases where you have created a curve using **Draw Spline**, you may want the curve to pass through a selection of those points currently lying away from the curve. You may force a straight line between two points by selecting the first of the two points in the **Point list** and then clicking on the **Draw straight to next point** button. This may have to be repeated for several consecutive points to achieve the desired curve.

8. Click on **OK** and save the curve. You can change the curve name from the default, **CreatedCurve**, and also the curve destination.

10.2.4 Measuring salt concentrations in the fractions

If you need to know the approximate conductivity or concentration of salt in your fractions, it is possible to calculate these from the conductivity curve.

Note: The conductivity signal is not linear more than up to 0.3 M, but you will still gain a relatively good idea of the salt concentrations above 0.3 M.

Stored in a chromatogram within a result file is a conductivity curve, usually given the name **Cond**. This curve represents the real conductivity data in mS/cm and should be used for calculations. Another curve, **Cond%**, is also present and is the same as **Cond** but re-scaled to display percentage values.

To make the calculations:

1. Select **Operations:Fraction histogram**. Select the **Cond** curve in the left list and the fractions curve should already be selected in the middle list. If you have earlier pooled fractions, it is possible to select the desired fraction curve. Click on **OK**.

2. Select the appropriate fraction curve in the **Chromatogram Layout** dialogue. In the active chromatogram you will see the fraction marks, the fraction histogram of the conductivity curve and any

other selected curves. Double click on the **XY** box to display the vertical cursor line. Click on the desired curve legend and the corresponding y-axis is displayed.

3. Use the mouse to drag the vertical bar back and forth along the x-axis. For a given fraction, its conductivity is displayed in the active **XY** icon box.

10.3 Automated evaluation procedures

An evaluation procedure is a recorded sequence of interactive operations in the Evaluation module, which can be executed for automated data evaluation and report generation. It can be used for single chromatograms and for a number of chromatograms in different result files. The concept is analogous to the "macro" facility provided in many word processing and other programs. Evaluation procedures can also be called from methods, making run execution, evaluation and documentation fully automatic. Automation is achieved using the **Procedures** menu.

A procedure can be recorded and run using the **Procedures** menu commands or from the commands available in the **Procedure Editor** dialogue. The **Procedure Editor** dialogue also allows you to view and edit the instructions within a procedure. The evaluation module is locked during a batch run.

10.3.1 Recording a procedure

1. Open the appropriate results file in the Evaluation module.

2. To begin recording a procedure select **Procedures:Record on**. The **Procedure Editor** dialogue is displayed in Record mode.

3. Minimize the **Procedure Editor** dialogue.

4. Perform the evaluation steps that the procedure is to contain. These steps are recorded as you perform them.

5. To stop the recording, either:

   - select **Procedures:Record off**, or
   - restore the iconised **Procedure Editor** dialogue and click on the '**stop**' button or select the dialogue **Control:End Record** menu command.

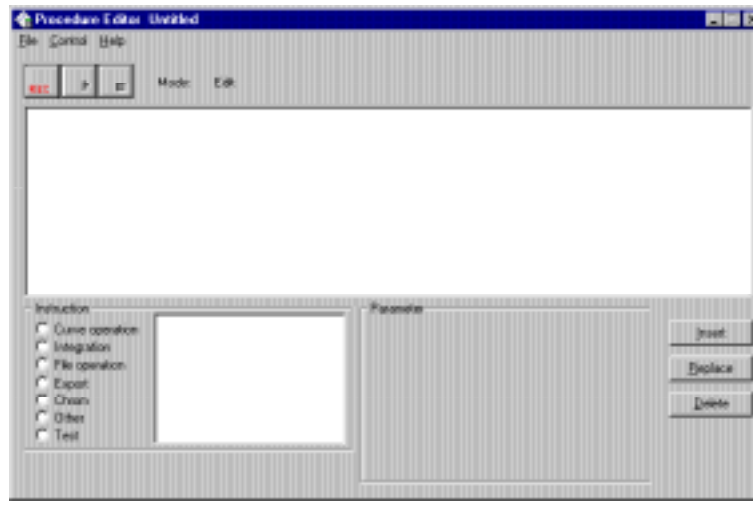


Figure 10-32. Procedure editor dialogue.

6. The recorded procedure can be viewed in the **Procedure Editor** dialogue. Restore the iconised dialogue if necessary.

7. More steps can be added to the evaluation procedure by repeating steps 2-6. The new steps are added to the previous procedure.

Note: New lines will be inserted into the procedure after the selected line in the currently listed procedure. This can be used to insert new instructions between existing instructions.

8. If required edit the evaluation procedure (see Section 10.3.2).

9. Select **File:Save** or **File:Save as** from the dialogue menu and enter a name for the procedure. The evaluation procedure is saved within UNICORN and is specific to your user name. If you have **Edit global list(s)** access you may also check the **Global procedure** option to make the procedure available to all users. Global procedures are marked with **[Global]** before the name. Even if the results of an evaluation session are not saved, the created evaluation procedure(s) are saved.

10. Choose dialogue **File:Exit** menu command.

Note: If you already have an existing procedure open for editing in the **Procedure Editor** dialogue (see Section 10.3.2) and you follow the above procedure, new instructions will be added to the currently open procedure, i.e. you will not be creating a new procedure.

To create a new procedure, select **File:New:Procedure** or **Procedure:Edit:New** to display the **Procedure Editor** dialogue. To begin recording, click on the **Rec** button or select the dialogue **Control:Record** menu command.

10.3.2 Editing an existing procedure

Evaluation operations are represented by instructions (see Appendix D.4) in the **Procedure Editor** dialogue. These may be modified to suit specific evaluation needs and be saved for later use.

Note: You are recommended to be restrictive in editing existing procedures. It is recommended to open an existing procedure and save a copy under a new name and use this copy to perform any editing procedures.

1. Click on **Procedures:Edit:Open** and select the desired evaluation procedure from the list. The **Procedure Editor** dialogue is displayed for the selected procedure.

2. To view the parameters contained within a specific instruction in the procedure, select it and view the information contained within the **Instructions** and **Parameters** fields of the dialogue.

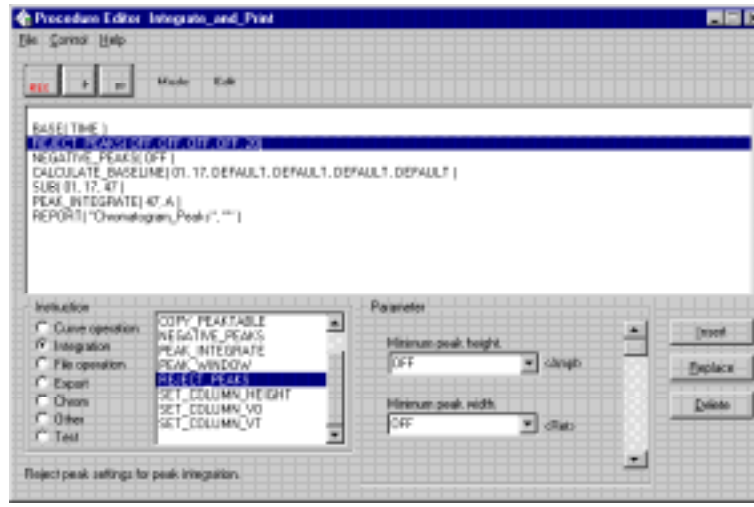


Figure 10-33. Editing procedures in the Procedure editor dialogue.

There are several types of instructions, as denoted by the options buttons, e.g. **Curve operation**, **Integration**, **File operation** etc., in the **Instructions** field. The appropriate option button and instruction therein will be automatically selected when you select an instruction in the procedure.

The specific parameters contained within the selected procedure instruction are displayed in the **Parameters** field.

A simple definition of the selected instruction is displayed at the bottom left-hand corner of the dialogue. A list of procedure instructions with fuller descriptions and parameters can be found in Appendix D.3.

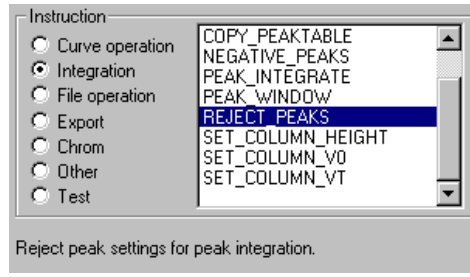


Figure 10-34. Instructions field in the Procedure editor dialogue.

3. To edit the parameters of a selected instruction, enter the new value in the appropriate place in the **Parameters** field. When you have made the desired changes, click on the **Replace** command button. You will now see that the selected instruction in the evaluation procedure is updated in accordance with the new parameters assigned to it.

4. You can insert new instructions *after* the currently selected procedure instruction. Select an instruction type and instruction in the **Instructions** field and enter the appropriate parameter values in the **Parameters** field. Click on the **Insert** command button. The new instruction will now be added to the evaluation procedure.

5. To remove an instruction from the evaluation procedure, select it in the procedure listing and click on the **Delete** button.

6. To save the edited procedure select **File:Save** or **File:Save as** from the dialogue menu commands. Name the procedure.

7. Choose **File:Exit** from the menu bar in the dialogue.

10.3.3 Renaming and removing procedures

Procedures that you have created can be renamed or deleted from the list of available procedures.

Note: You are recommended to be restrictive in the renaming and removing of existing procedures.

Renaming a procedure

1. Select **Procedures:Edit:Rename**.

2. Select a procedure in the **Rename Procedure** dialogue and enter a new name in the **New name** field.

3. Click on **OK**.

Removing a procedure

1. Select **Procedures:Edit:Delete**.

2. Select a procedure in the **Delete Procedure** dialogue and enter a new name in the **New name** field.

3. Click on **OK**.

10.3.4 Points to watch

In recording and editing evaluation procedures for automatic evaluation, beware of the following potential pitfalls:

- Make sure that the procedure addresses the right curves. Curves are identified by storage position alone: thus the instruction ADD (01,02,03) will try to add curve 01 to curve 02 and store the result in 03, regardless of the contents of 01 and 02. If 03 contains a curve which is not a raw data curve, the existing curve in 03 will be overwritten. If 03 contains a raw data curve, the procedure will stop with an error message. The raw data curves will always occupy the same positions for a given strategy, e.g. UV in position 01. If the operation is not valid when the procedure is run, the procedure will stop at the instruction with an error message. Any subsequent instructions in the procedure will not be executed.
- In calculating a baseline, UNICORN suggests default values for the four control parameters (see Section 10.1.2) based on the appearance of the curve. To instruct UNICORN to use default values appropriate for the curve every time the procedure is run, choose the Default setting in the appropriate fields for the parameters. For example;

CALCULATE_BASELINE (01, 06, XXX, XXX, XXX, XXX)

can be changed to:

CALCULATE_BASELINE (01, 06, DEFAULT, DEFAULT, DEFAULT, DEFAULT)

10.3.5 Running evaluation procedures

To run a procedure for a specific chromatogram, first make sure that the desired chromatogram is active. Click on **Procedures:Run** and choose the desired evaluation procedure. Click on **OK** and the procedure runs at once. You can also open the **Procedure Editor** dialogue and select the dialogue **Control:Run** menu command or click on the 'Play' button.

10.3.6 Batch runs

It is possible to apply an evaluation procedure to a designated batch of result files even if they are not open on the Evaluation workspace. It is especially useful, for example, to perform integration with the same parameter settings on many results, or to print a number of results with the same settings. The batch run is done in the background of the Evaluation module and thus the results of the run are not seen. You will, of course, receive any print-outs or report documentation if this was one of the steps in the run procedure (see Section 9.5.3).

1. Select **Procedures:Batch run** and the **Open Procedure** dialogue is displayed. Select the evaluation procedure and click on **OK**. The **Batch Run** dialogue, which allows you to search for the result files and/or chromatograms on which you wish to perform the batch run.

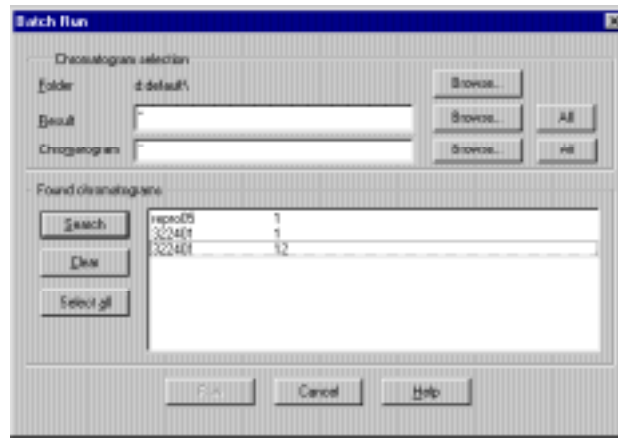


Figure 10-35. Batch run dialogue.

2. The search will take place in the displayed folder only. To select another folder, click on the **Browse** button and open the desired folder.

3. The search will take place in all result files as denoted by the asterisk '*****', within the selected folder. You can instead select a specific result file using the **Browse** function. Moreover, you can use wildcard characters to search within result files with a specific name profile.

   You can use standard wildcard characters in the file name specification (* stands for any number of characters and ? for any single character). For example:

iex	will search files named iex
iex*	will search all files with names beginning iex
*iex	will search all files with names ending iex
?iex	will search only 4-character names ending in iex

   To return to the default setting to search in all result files, click on **All**.

4. By default, all chromatograms are searched for within the designated folder and result files, as denoted by the asterisk '*****'. As for result files you can select a specific result file using the **Browse** function or you can use wildcard characters to search for chromatograms with a specific name profile.

   To return to the default setting too search for all chromatograms, click on **All**.

5. Click on the **Search** button and a list of chromatograms will be displayed based on the designated search criteria. A new search can be performed with new search criteria without erasing the first found chromatograms from the list.

6. Select the chromatograms that you want to import. If you click on the **Select All** button, all of the displayed chromatograms are selected. If you want to clear the list of displayed chromatograms, click on **Clear**.

7. Click on **Run** to perform the batch run. Any created curves and peak tables will be saved in each result file automatically.

8. To view the results of a batch run on a specific result/ chromatogram, open this in the Evaluation workspace.

Note: If you include the currently opened result file in the batch run, it will stop temporarily and you may exclude this result file from the batch run. If you keep the current result file in the batch run, the result file will be saved and then re-opened to ensure that the contents of the Evaluation workspace reflects the results of the evaluation procedure. The subsequent result files in the batch run will always be processed automatically.

10.3.7 Evaluation procedures and reports

The creation of evaluation procedures, combined with batch runs, is a very useful tool to produce printed documentation simultaneously for many result files, e.g. for a number of scouting runs. This removes the necessity to open/import result files onto the Evaluation workspace.

1. Begin recording a new procedure by selecting **Procedures:Record on**.

2. Select **File:Report** and choose a report format (see Section 9.5.3).

3. Select **Print** in the **Generate Report** dialogue as the final instruction.

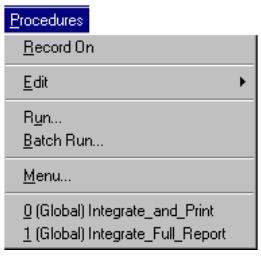
4. Stop the record function by selecting **Procedures:Record Off**.

5. Save the procedure.

6. Now do a batch run (see Section 10.3.5) on all the desired result files to get the printed reports. The procedure can also be saved with a method to get automatic printouts at the end of a run (Section 5.6.5).

Note: If the selected report format is changed in **File:Report** the new format will be applied when the procedure is run. If the format is subsequently deleted, the procedure cannot be run.

10.3.8 Placing a procedure on the menu and running



It is possible to choose a maximum of 15 created evaluation procedures to be placed onto the **Procedures** menu.

Select **Procedures:Menu** and select the evaluation procedure to be added to the menu. Click on **OK**. Activate a chromatogram and select the **Procedures** menu. You will see the procedure that you added to the menu. Select this procedure and it is automatically run for the active chromatogram. The menu addition is remembered in UNICORN even if the results of the current evaluation session are not saved.

To take away a procedure from the menu, deselect it from the **Procedures:Menu** list.

10.3.9 Exporting data or curves

Data and curves can be exported to other file formats. Select **File:Export** and then the appropriate cascade menu choice. Alternatively, the **Edit:Copy to clipboard** function can be used, which is the quickest and easiest way to copy a chromatogram into, for example, a spreadsheet application software.

10.3.10 Exporting results

Data can be exported to ASCII (text) and Lotus 1-2-3™ (WKS) spreadsheet formats. Select the format that you find to be most useful together with your other application software. Peak tables are exported as text strings in the ASCII format, but as numerical values in the Lotus 1-2-3 format. When exporting peak tables, all possible columns in the table are exported.

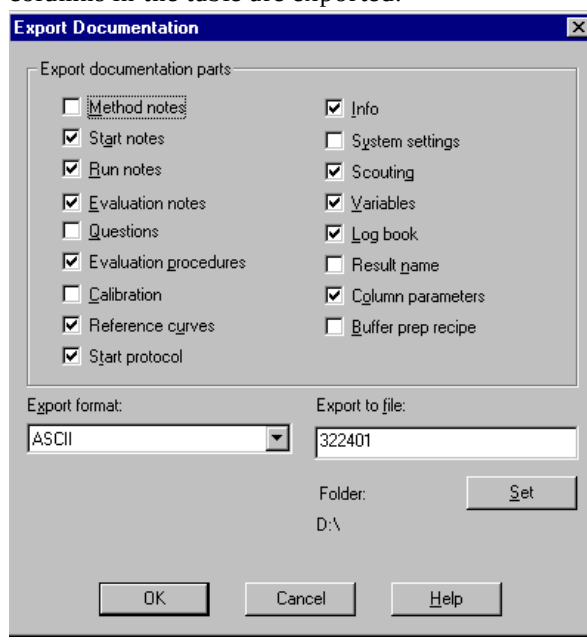


Figure 10-36. Export Documentation dialogue.

Curves are exported as two series of numerical co-ordinates referring to time/volume and signal respectively. Only one curve can be exported in each file. When exporting a curve, you may choose to export only a portion of the curve by inserting the limiting retention values directly into the boxes in the **Cut curve** field or by visually selecting the part of the curve using the **Cut graphically** option. You

may also reduce the number of data points that are exported. For example, reducing the number of data points by a factor of five will export every fifth point to the file.

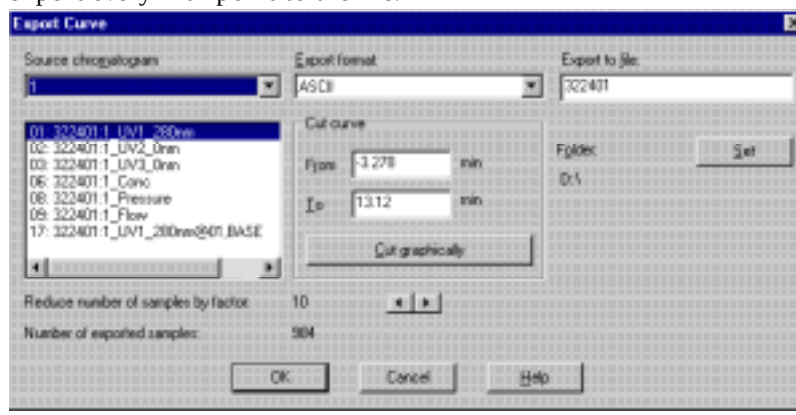


Figure 10-37. Export Curve dialogue.

The suggested name is the same as the name of the current file. You may change the file name if desired. The extension of the exported file will be .ASCII or .WKS depending on the file format. By default, files are stored on the root folder on the drive shown in the Folder field. It is possible to change the destination by pressing the Set command button.

10.3.11 Copying results to the clipboard

A related activity is **Edit:Copy to Clipboard** where the contents of the active window are copied to the Windows clipboard and can be later easily pasted into other programs, e.g. Microsoft® Word™. Curves and Documentation are copied as Windows enhanced metafiles (.emf), while Peak tables are copied as text. In the latter case, only the columns selected in the **Chromatogram Layout** dialogue will be copied.

Introductory material

Methods and runs

Evaluation

System management

Appendices

11 Security features

This chapter considers security features in UNICORN, under the topics

- access security
- system connection security
- data security
- security recommendations for control stations

11.1 Access security

Operation of UNICORN is restricted to authorised users, defined in the system by the system administrator (see Section 14.3). User access may be protected by a password. The minimum password length for users is defined when UNICORN is installed (see Section 13.4). In installations where access security is important, passwords should be enforced and should be changed regularly (see Section 14.3.2).

Each user is assigned an access level and a folder access profile, defining the operations that the user can perform and the folders the user is allowed to access (see Section 14.3). For system security, administrative routines such as user and system definition should be permitted only to the system administrator.

Installation of UNICORN establishes a default user with full access rights in the system. It is important for system security that this user is deleted when the site-specific users have been created.

11.2 Connection security

To prevent conflicts in system control requests, each system in an installation may have only one control mode connection at any one time.

A running system may be left in a view mode connection and locked with a password (independent of the user's logon password) to prevent other users from establishing a control mode connection and interfering with controlling the separation process. If the system is left unlocked in a view mode connection, any user may establish a control mode connection to the system.

We recommend that systems are always locked when a user leaves the system. For controlled and locked systems, the responsible user is identified in the system control window for view mode connections. A system which is left unlocked with no control mode connection has no identified responsible user.

Systems may be locked even when they are idle, to allow users to reserve a system for later use.

11.3 Data security

11.3.1 Network communication failure

If the network communication fails while a method is running, any control mode connection on a remote station will lose control of the system and results destined for network drives cannot be saved in their correct folders. In this event, the run continues under control from the local station. Results are saved on the local station with the original result file name in the folder DRIVE:\Unicorn\Local \Fil\Failed, from which they can be retrieved after the run is complete. To retrieve results from the Failed folder:

1. Start UNICORN (if it is not already started) on the local station where the separation process was run.

2. Log on as a user with access to the Failed folder.

3. You can now open the result file in the evaluation module and move it to a suitable location on the network server when network communication is re-established so that it is accessible from remote stations.

Note: Result files are saved directly under the Failed folder, and are identified by result file name. Files with the same result file name base are distinguished by an incremental serial number, in the same way as result files in any other folder.

Access to the Failed folder

The system administrator may choose one of two policies concerning access to the Failed folder:

- Grant access to the Failed folder to all users, so that each user can retrieve his or her own result files. This places the responsibility for retrieving result files and deleting old files from the Failed folder on the individual users. Note that with this policy any user will be able to examine, copy, move and delete the other users'

results in the Failed folder. This policy has the advantage that the Failed folder can conveniently be used to temporarily store methods and results from runs performed from the local station when the network is not running.

- Grant access to the Failed folder only to one or a few users, who share the responsibility for retrieving results files and deleting old files from the Failed folder. This policy should be used if the installation requires restricted access to users' result files. Note that the user(s) with access to the Failed folder should also have access to other users' home folders, to be able to copy or move result files to suitable destinations.

In granting access to the Failed folder, it is sufficient to grant access to DRIVE:\Unicorn\Local\Fil\Failed, since this is the path to the Failed folder on each local station.

11.3.2 Local station failure

If the local station fails during a run, the run may continue but the results generated after the failure cannot be saved. An autosave feature saves a temporary result file on the local station during runs at a pre-set interval defined in the system definition (see Section 14.1). When the local station is restarted, the temporary result file will be transferred to the original result file destination (or to the Failed folder if the original destination was on a network drive which is not currently available). The result file will contain the results of the run up to and including the last autosave time before the failure. Results after this time will be lost.

11.4 Security recommendations for control stations

Chromatography systems may be controlled without running the user interface modules. In a network installation, this situation can arise if a system is controlled from a remote station without starting UNICORN on the local station. In a stand-alone installation, the situation can arise if a user quits UNICORN after starting a run. In both cases, it is not apparent from the desktop that UNICORN control software is actually running, and there is therefore a risk that Windows NT may be shut down and the computer turned off in the belief that it is not in use. To prevent this:

- Do not quit UNICORN if you are controlling a system.
- Do not turn off local station computers in a network installation.
- If possible, start UNICORN application program on all local stations in a network installation and establish a view mode connection, as an indication that a connected system might be running.

12 Network setup

UNICORN 3.00 can be installed in a network environment, allowing chromatography systems to be controlled from any PC in the network where UNICORN is installed. This chapter describes how to set up the network environment before installing UNICORN. This chapter is only relevant if you will be running UNICORN to control chromatography systems remotely.

The Network setup should be done by someone with experience in Windows NT and network installations.

12.1 Introduction

The different network setups that UNICORN has been tested in are the following:

- NT domain with TCP/IP
- NT workstations connected to a Novell server

Note: The same version of UNICORN must be installed on both the computers with the chromatography system and the remote control computers. Mixing different versions will not work.

Establishing and maintaining a networked UNICORN installation requires some understanding of the working of the NT operating system and the concept of NT domains if used. We recommend that a competent network administrator, whom will also be involved in the installation of UNICORN software, maintain the network.

The network administrator is not necessarily the same person as the system administrator for UNICORN. Once the network is set up, network functions are entirely transparent to UNICORN users, and the network administrator does not need to understand the use of UNICORN for controlling chromatography systems.

Security is very important in a networked UNICORN installation. Three aspects of security can be distinguished, and the responsibility for maintaining security is shared by the network administrator and the system administrator for UNICORN:

	Network administrator	*UNICORN administrator*
Data storage security (back-up routines)	Back-up routines for server and local disks.	Controlled user access to home and shared folders, e.g. placing all home folders on a shared disk to prevent data from being scattered throughout the network.
Network access security	Maintenance of user passwords and access rights to shared resources.	-
UNICORN access security	-	Maintenance of user profiles

PC's with UNICORN software can be categorised as follows:

- A local station is a PC to which chromatography systems are physically connected. UNICORN software must be installed on all local stations in the network.
- A remote station is a PC to which no systems are physically connected, but which can control systems over a network link. UNICORN software is installed with the **Remote Only** option on remote stations.

Chromatography systems in the network can be controlled from either remote or local stations. A local station in a network can also be used as a remote station to control other systems.

Note: If a run is being controlled from a remote station and a network communication error occurs, the run will continue under the control of the local station. Results will be saved in the Failed folder on the local station. A control mode connection can be established on the local station to control the run.

For a chromatography system to be accessible in the network, the local station must be switched on and logged on to the network. The user interface for UNICORN does not however need to be started on the local station. System control from a remote station is managed through network level routines, which are started at log on time on the local station.

A local station can be used to control the chromatography system directly connected to the PC without logging on to the network. (The station must however have been logged on to the network once previously so that the necessary files are copied from the network server to the local computer.) This copying is performed automatically. Method and result files stored on the network drives will of course not be accessible. For runs performed in this stand-alone mode where the result file is directed to a network drive, the results will be saved in the Failed folder on the local station.

Note: It is still required that the user log on to the local Windows NT machine using a valid user ID.

12.2 Requirements

The following are minimum network requirements for running UNICORN in a network installation:

- Windows NT workstation version 4.0.
- 3Com Etherlink III PCI network card or a Compaq NetFlex-3 network card.
- A valid network connection.
- Protocols and services such that named pipes are usable over the network.
- Protocols and services such that folders can be connected to a drive unit.

The two last points are satisfied if the services; Server, Client and NetBIOS are installed and one of the protocols TCP/IP or NWLink IPX/SPX.

12.3 Installation guide

12.3.1 TCP/IP - NT domain

This is an example of how to setup the network so that UNICORN may run across the network when the network protocol is TCP/IP and there is an NT domain controller.

The setup of the NT domain controller is not described here; a competent network administrator is generally needed to setup an NT domain server.

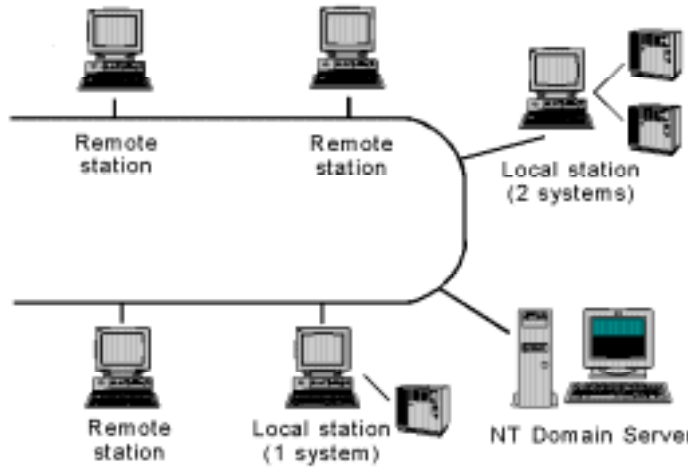


Figure 12-1. General network organisation based on a Windows NT domain controller.

Creating user groups and users of UNICORN

This is performed by the network administrator:

1. Log on to the NT domain controller with administrative rights.

2. Enter the **User Manager** for the domain by selecting Start:Programs:Administrative Tools (Common).

3. Create a group that all UNICORN users will be part of. Select the **User** menu and the **New Local or Global Group** item.

4. In the dialogue that is displayed, enter a suitable name for the group and click on **OK**.

5. Create the users that will run UNICORN. Select the **User** menu and the **New User** item.

6. In the dialogue that is displayed, enter user name (maximum 20 characters and must be unique within the domain), description and password. Click on the **Groups** button.

7. In the dialogue that is displayed, select the newly created group of UNICORN users and click on the **<- Add** button. Finally click on the **OK** button in the **Group** membership dialogue.

8. Click on the **OK** button in the **New User** dialogue and the user is created.

9. When all users have been created, click on **Close** in the **New User** dialogue.

Sharing a folder

This is performed by the network administrator:

1. Log on to the NT computer designated to be the UNICORN file server with administrative rights.

2. Start Windows NT Explorer with Start:Programs:Windows NT Explorer.

3. Open the right mouse button menu for the folder that will serve as the UNICORN server disk. Select **Sharing**.

4. In the dialogue that is displayed, click on the **Shared As** radio button and enter a suitable share name, e.g. UNICORN. This name will be used when connecting to this folder.

5. Set up the permissions for the shared folder by clicking the **Permissions** button. Select **Everyone** in the list box and click on the **Remove** button. Click on the **Add** button and in the dialogue that is displayed make sure that the domain is selected in the top drop-down list. Select the newly created user group in the list and click on the **Add** button and finally select **Full Control** in the bottom drop-down list and click on the **OK** button.

6. Select **OK** to accept Share in the **Type of Access** dialogue.

Install TCP/IP on the computer

This is performed by the network administrator:

1. Open the control panel with Start:Settings:Control Panel.

2. Double click on the **Network** icon.

3. A question will be displayed asking if you want to install Windows NT Networking. Click on **Yes**.

4. In the next dialogue select **Wired to the network** and click on **Next**.

5. Specify the network adapter to use by clicking on **Select from list**.

   If your adapter is shown in the displayed list, select it and click on **OK**.

Alternatively click on **Have disk** and insert the disk with the latest version of your driver. Enter the path to it in the dialogue and click on **OK**.

6. Click on **Next**.

7. Make sure your selected adapter is shown in the list and click on **Next**.

8. Select **TCP/IP** as the protocol to use.

9. Click on **Next**.

10. Select all default the services and click on **Next**.

11. Configure the TCP/IP protocol. Information specific to the network will have to be entered. This information should be supplied by the network administrator. Click on **Next** when ready.

12. Click on **Next** again to install the selected components.

13. Insert the Windows NT CD-ROM disk and enter the path to it in the dialogue (Example: **D:**). Click on **Continue**.

14. A dialogue is displayed, displaying where the files will be read. Click on **Continue**.

15. The bindings are displayed. Click on **Next** to continue.

16. Click on **Next** to start the network.

17. On the primary Domain Controller, run Server Manager by selecting Start:Programs:Administrative Tools:Server Manager.

18. From the menu select **Computer** and then the **Add to Domain** item.

19. Select the **Windows NT** or **Server** radio button and enter the NT workstation name in the **Computer Name** field. Click on **Add**. The computer is added to the domain.

20. On the Windows NT workstation select the **Domain** radio button and enter the name for the domain. The network administrator should supply this name. Click on **Next** to continue. You will be welcomed to the domain.

21. Acknowledge and click on **Finish**.

22. Select the option to restart your computer now. The computer is restarted.

23. Log on to the Domain with administrator rights.

Installing TCP/IP on the computer

This is relevant when network has been installed earlier.

1. Log on to the computer with administrator rights.

2. Open the control panel with Start:Settings:Control Panel.

3. Double click on the **Network** icon.

4. In the **Network** dialogue, enter the **Adapters** tab and click on the **Add** button.

5. In the dialogue that is displayed select the correct driver for your network adapter and click on the **OK** button. Click on the **Have disk** button and insert the disk with the latest version of your driver and enter the path to it in the dialogue.

6. In the **Network** dialogue, enter the **Services** tab and click on the **Add** button.

7. In the dialogue that is displayed, select **NetBIOS** and click on the **OK** button.

8. Click on the **Add** button again.

9. In the dialogue that is displayed, select **Server** and click on the **OK** button.

10. Click on the **Add** button again.

11. In the dialogue that is displayed, select **Workstation** and click on the **OK** button.

12. In the **Network** dialogue, enter the **Protocols** tab and click on the **Add** button.

13. In the dialogue that is displayed, select **TCP/IP Protocol** and click on the **OK** button.

14. In the **Network** dialogue click on the **OK** button. When the window is writing this new data to the hard disk the configuration dialogues for the TCP/IP protocol will be displayed. Information specific to the network must be entered. This information should be supplied by the network administrator.

Installing UNICORN

1. Start Windows NT Explorer with Start/Programs:Windows NT Explorer.

2. Connect the shared folder on the UNICORN file server with the designated drive letter. You can do this by selecting Tools:Map Network Drive).

3. In the dialogue that is displayed select the drive letter in the upper drop-down list.

4. If the current user ID does not have permissions to the shared UNICORN folder on the server, enter a valid domain user ID in the **Connect As** field.

5. In the **Shared folders** list at the bottom of the dialogue the network can be seen, double click on the server with the shared folder.

6. Available shared folders on the selected server are displayed. Double click on the shared folder UNICORN will use.

7. A dialogue appears with a request to give a valid user name and password. The user name will be the ID previously entered in the **Connect As** field. Give the password and click on **OK**.

8. Install UNICORN (see Chapter 13), select **Network** version.

9. Re-boot the PC and log on as one of the domain users that will run UNICORN.

10. Connect the shared folder again, this is necessary since each user has his own connected network drives. Repeat steps 1, 2, 3,5 and 6 above and make sure the **Reconnect at Logon** option is checked.

11. Start UNICORN and set up system definitions and user profiles (see Chapter 13).

12.3.2 IPX/SPX - Novell server

This is an example of how to set up the network so that UNICORN can run across the network when the network protocol is IPX/SPX and uses a Novell server.

The general network organisation is described in the following picture.

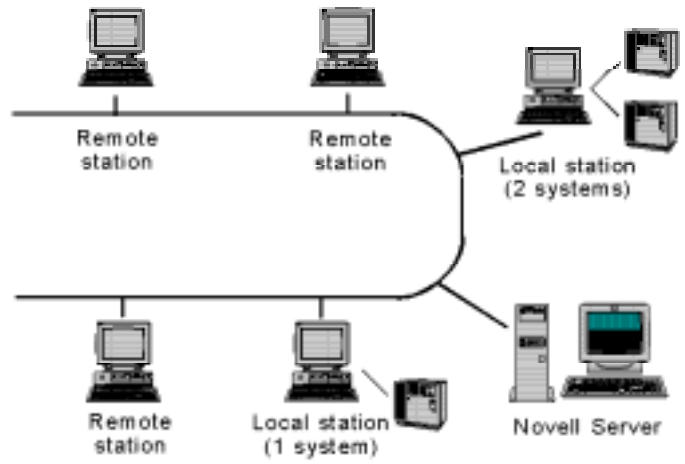


Figure 12-2. General network organisation based on a Novell network controller.

The setup of the Novell server is not described here; a competent network administrator is normally needed to setup a Novell server.

Creating user group and users of UNICORN on the Novell server

This is performed by the network administrator.

The network administrator creates the users that will be running UNICORN on the Novell server. The procedure for this is not described here.

Sharing a folder

This is performed by the network administrator.

The network administrator creates shared folder on the Novell server which UNICORN will use for shared data. The procedure for this is not described here.

Creating user group and users of UNICORN on the local computers

1. Log on to the client computer with administrator rights.

2. Enter the **User Manager** with Start:Programs:Administrative Tools (Common):User Manager.

3. Create the users that will run UNICORN. Select the **User** menu and the **New User** item.

4. In the dialogue that is displayed, enter the user name (the UNICORN users created by the network administrator), description and password (enter the password the user has in the Novell network). Make sure that the **Password Never Expires** option is checked and that the **User Must Change Password at Next Logon** option is not checked.

5. Click on **OK** in the **New User** dialogue and the user is created.

6. When completed, close **User Manager**.

Installing IPX/SPX on the computer

1. Open the control panel with Start:Settings:Control Panel.

2. Double click on the **Network** icon.

3. A question will be displayed asking if you want to install Windows NT Networking. Click on **Yes**.

4. In the next dialogue select **Wired to the network** and click on **Next**.

5. Specify the network adapter to use by clicking on **Select from list**.

   If your adapter is shown in the displayed list, select it and click on **OK**.

   Alternatively click on **Have disk** and insert the disk with the latest version of your driver. Enter the path to it in the dialogue and click on **OK**.

6. Click on **Next**.

7. Make sure your selected adapter is shown in the list and click on **Next**.

8. Select **NWLink IPX/SPX Compatible Transport** as the protocol to use. Deselect **TCP/IP**.

9. Click on **Next**.

10. Select all default the services and click on **Next**.

12. Select **Client Service for NetWare** and click on **OK**.

13. Click on **Next**.

14. Click on **Next** again to install the selected components.

15. Insert the Windows NT CD-ROM disk and enter the path to it in the dialogue (Example: **D:**). Click on **Continue**.

16. A dialogue is displayed, displaying where the files will be read. Click on **Continue**.

17. The bindings are displayed. Click on **Next** to continue.

18. Click on **Next** to start the network.

19. Select the **Workgroup** radio button and enter a name for the workgroup. The network administrator should supply this name, click on **Next** to continue.

20. Click on **Finish**.

21. Remove the CD-ROM disk.

22. Select the option to restart your computer now. The computer is restarted.

23. Log on with administrator rights.

24. The **Select NetWare Logon** dialogue is displayed. The network administrator must supply the **Default Tree and Context** to use. Select the **Default Tree and Context** radio button, enter the information from the network administrator and click on **OK**.

25. Since the name and password of the user you logged on as are not a valid account on the Novell server, a message will be displayed after a 30 second timeout, **NetWare Authentication Failure**. Click on **Yes** to keep the default tree and context.

Installing UNICORN

1. Start Windows NT Explorer with Start/Programs:Windows NT Explorer.

2. Connect the shared folder on the UNICORN file server with the designated drive letter. You can do this by selecting Tools:Map Network Drive).

3. In the dialogue that is displayed select the drive letter in the upper drop-down list.

4. Enter a valid domain user ID in the **Connect As** field.

5. In the **Shared folders** list at the bottom of the dialogue the network can be seen, double click on the Novell server with the shared folder.

6. Available shared folders on the selected server are displayed. Double click on the shared folder UNICORN will use.

7. A dialogue appears with a request to give a valid user name and password. The user name will be the ID previously entered in the **Connect As** field. Give the password and click on **OK**.

8. Install UNICORN (see Chapter 13), select **Network** version.

9. Re-boot the PC and log on as one of the users that will run UNICORN.

10. The **Select NetWare Logon** dialogue will be displayed. The network administrator must supply the **Default Tree and Context** to use. Select the **Default Tree and Context** radio button, enter the information from the network administrator and click on **OK**.

11. Connect the shared folder again, this is necessary since each user has his own connected network drives. Repeat steps 1, 2, 3,5 and 6 above and make sure the **Reconnect at Logon** option is checked.

12. Start UNICORN and set up system definitions and user profiles (see Chapter 13).

13 Installation

This chapter describes how to install UNICORN software.

13.1 Installation summary

The following installation procedures are required before UNICORN systems can be used:

1. Back up files if migrating from UNICORN 2.30 to UNICORN 3.00.
2. Set up the network environment (for network installations only).
3. Install UNICORN hardware and software.
4. Define access levels for the installation.
5. Define users with home folders and access profiles.
6. Check the system settings for the attached systems.

13.2 Migrating from UNICORN 2.30 to UNICORN 3.00

13.2.1 Before migration

CAUTION!
Before commencing with the migration from UNICORN 2.30 to UNICORN 3.00, ensure that all method files, MethodQueue files and result files are backed up on separate drive or medium and are separate to the UNICORN folders used on the network. When you access these files for the first time following installation of UNICORN 3.00, these will be reformatted so that they can be used by the new system.

- If you are using a system other than a standard ÄKTAdesign system, make sure to locate the strategy and template diskette supplied with your system.
- Document your user setup, access rights and folder structure so that they can be redone in the new version.
- Print out the system settings and calibration constants.
- Document the columns that have been added.

13.2.2 Migration and post-installation setup

1. Install UNICORN 3.00.

2. Install strategies either from diskette supplied with the original system using the **Have Disk** option, or by selecting from the standard systems supplied in the installation CD-ROM.

3. Set up the users, folders and access rights

4. Set up the columns.

5. Recalibrate the system.

6. Enter system settings.

7. Copy the methods and results you want using **Copy from External**.

8. If you are using multiple systems you might need to associate your copied files with a system in this process.

13.3 System requirements

For the hardware, software and network requirements, please refer to Appendix A.

13.4 Hardware installation

In most cases, an installation of your system will be performed by Amersham Biosciences authorised personnel. If your system is not pre-installed, then follow the steps below to install the expansion card in your PC. This procedure is only required in stand-alone installations, i.e. systems not connected to a network, and on local computers in network installations.

1. Turn off the power to your PC and remove the power cable from the mains socket.

2. Open the PC cover. Refer to your PC documentation if you are not sure how to do this.

3. Locate an empty full length expansion slot.

4. Take the expansion card out of the anti-static bag. Handle the card by its edges, and avoid touching the electronic components as far as possible, because discharges of static electricity can permanently

damage electronic components on the card. If you are working in a room where static electricity tends to build up, discharge any electricity from your body by touching an earthed metal surface (e.g. a water tap or radiator) before handling the card.

5. Two settings that are made on the expansion cards are for interrupt (IRQ) and address. These settings must be entered in the UNICORN software installation so that UNICORN can find the systems when started. To see which settings the card has, look at the card and compare it with the tables below. The IRQ is set by a jumper and the address is set with a dip-switch. Write down the settings on a paper for future use.

   When you are installing the UNICORN software and the setup program asks for the card settings, enter the correct values for IRQ and address. If for some reason the default settings can not be used on a specific computer, the settings have to be changed.

   CU 900 (ÄKTA systems)

   Alternative IRQs for this card are 3, 4, 5, 7, 10, 11, 12 and 15. The manufacturing default setting is IRQ5.

   The address is made up of two parts, the base address and the node address. Valid base addresses for this card are any hex address between 0 and FF0, the node address can be altered between 1-4. The manufacturing default setting for the address is 220 (base address 220 and node address 1). To change the settings for the address, refer to the tables

Node address	*Base address*	*S1:8*	*S1:7*
1	Base address + 0	ON	ON
2	Base address + 400h	ON	OFF
3	Base address + 800h	OFF	ON
4	Base address + C00h	OFF	OFF

Base address	S1:6	S1:5	S1:4	S1:3	S1:2	S1:1
100	ON	OFF	ON	ON	ON	ON
110	ON	OFF	ON	ON	ON	OFF
120	ON	OFF	ON	ON	OFF	ON
130	ON	OFF	ON	ON	OFF	OFF
140	ON	OFF	ON	OFF	ON	ON
150	ON	OFF	ON	OFF	ON	OFF
160	ON	OFF	ON	OFF	OFF	ON
170	ON	OFF	ON	OFF	OFF	OFF
180	ON	OFF	OFF	ON	ON	ON
190	ON	OFF	OFF	ON	ON	OFF
1A0	ON	OFF	OFF	ON	OFF	ON
1B0	ON	OFF	OFF	ON	OFF	OFF
1C0	ON	OFF	OFF	OFF	ON	ON
1D0	ON	OFF	OFF	OFF	ON	OFF
1E0	ON	OFF	OFF	OFF	OFF	ON
220	OFF	ON	ON	ON	OFF	ON
230	OFF	ON	ON	ON	OFF	OFF
240	OFF	ON	ON	OFF	ON	ON
250	OFF	ON	ON	OFF	ON	OFF
260	OFF	ON	ON	OFF	OFF	ON
280	OFF	ON	OFF	ON	ON	ON
290	OFF	ON	OFF	ON	ON	OFF
2A0	OFF	ON	OFF	ON	OFF	ON
300	OFF	OFF	ON	ON	ON	ON
310	OFF	OFF	ON	ON	ON	OFF
320	OFF	OFF	ON	ON	OFF	ON

330	OFF	OFF	ON	ON	OFF	OFF
340	OFF	OFF	ON	OFF	ON	ON
350	OFF	OFF	ON	OFF	ON	OFF
3E0	OFF	OFF	OFF	OFF	OFF	ON

UNICORN Control Board, AT (BioPilot systems)

Alternative IRQs for this card are 3, 5, 10 and 11. The manufacturing default setting is IRQ5.

Alternative addresses for this card are shown in the table. The value that should be entered as an address in the UNICORN software installation is the first number listed in the Address DualPort column. The manufacturing default setting for the address is no. 10 and for this setting C8000 should be entered as the address in the UNICRN software installation.

No.	S1:4	S1:3	S1:2	S1:1	Address DualPort	Address IRQ
0	ON	ON	ON	ON	0A0000 - 0A1FFF	0A2000 - 0A3FFF
1	ON	ON	ON	OFF	0A4000 - 0A5FFF	0A6000 - 0A7FFF
2	ON	ON	OFF	ON	0A8000 - 0A9FFF	0AA000 - 0ABFFF
3	ON	ON	OFF	OFF	0AC000 - 0ADFFF	0AE000 - 0AFFFF
4	ON	OFF	ON	ON	0B0000 - 0B1FFF	0B2000 - 0B3FFF
5	ON	OFF	ON	OFF	0B4000 - 0B5FFF	0B6000 - 0B7FFF
6	ON	OFF	OFF	ON	0B8000 - 0B9FFF	0BA000 - 0BBFFF
7	ON	OFF	OFF	OFF	0BC000 - 0BDFFF	0BE000 - 0BFFFF
8	OFF	ON	ON	ON	0C0000 - 0C1FFF	0C2000 - 0C3FFF

9	OFF	ON	ON	OFF	0C4000 - 0C5FFF	0C6000 - 0C7FFF
10	OFF	ON	OFF	ON	0C8000 - 0C9FFF	0CA000 - 0CBFFF
11	OFF	ON	OFF	OFF	0CC000 - 0CDFFF	0CE000 - 0CFFFF
12	OFF	OFF	ON	ON	0D0000 - 0D1FFF	0D2000 - 0D3FFF
13	OFF	OFF	ON	OFF	0D4000 - 0D5FFF	0D6000 - 0D7FFF
14	OFF	OFF	OFF	ON	0D8000 - 0D9FFF	0DA000 - 0DBFFF
15	OFF	OFF	OFF	OFF	0DC000 - 0DDFFF	0DE000 - 0DFFFF

6. Close the cover on your PC.

7. Connect the card to the liquid handling module CU connector using the communication cable provided. Additional liquid handling modules up to a total of four may be connected.

13.5 Software installation

UNICORN will normally be installed by your Amersham Biosciences representative. Follow the instructions below if you need to install the program yourself. If the system is connected to the network and installed to support remote control, make sure that the same version of UNICORN is installed on all stations in the network.

13.5.1 Installing UNICORN for the first time

The installation procedure described below assumes that the operating system Windows NT is correctly installed on your computer. Refer to the operating system documentation for details. For network installations, the network must be correctly set up.

UNICORN is supplied on a CD-rom. Files on the CD-rom are compressed and cannot simply be copied onto the hard disk. The installation procedure described below creates the required folder structure on the hard disk and decompresses the files. Do not attempt to decompress the distribution files using any other file decompression utility.

Follow the procedure below to install UNICORN. For a network installation, follow this procedure on each computer in the network. You can quit the installation at any point by clicking on either the **Cancel** button or the **Exit** button. If you do this, however, the installation will be incomplete and the software cannot be used.

Note: Before the installation is performed on a local station be sure that you know which interrupt and address that the system(s) will use. It is possible to install the UNICORN software without this information, but the system(s) will not work. Refer to chapter 13.3 for information about the system settings.

1. For network installations, log on to the network and check that you have access to the server disk and folder where UNICORN network components are to be installed.

2. Insert the CD-rom disk into the CD-rom drive.

3. Select Run from the taskbar Start button menu.

4. Write the command `d:setup`, where `d:` is the unit for your CD-rom drive. Click on **OK**.

5. The UNICORN setup program is launched and you will see the **Welcome** dialogue. Click on **Next** to continue.

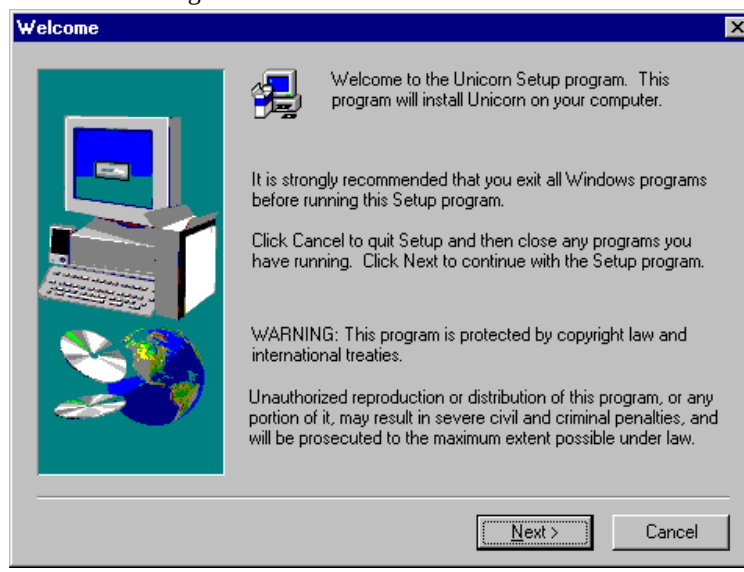

Welcome
Welcome to the Unicorn Setup program. This program will install Unicorn on your computer.
It is strongly recommended that you exit all Windows programs before running this Setup program.
Click Cancel to quit Setup and then close any programs you have running. Click Next to continue with the Setup program.
WARNING: This program is protected by copyright law and international treaties.
Unauthorized reproduction or distribution of this program, or any portion of it, may result in severe civil and criminal penalties, and will be prosecuted to the maximum extent possible under law.
Next >
Cancel

Figure 13-1. Welcome dialogue

6. The license agreement must be accepted to install UNICORN. Click on **Yes** to continue the setup program.

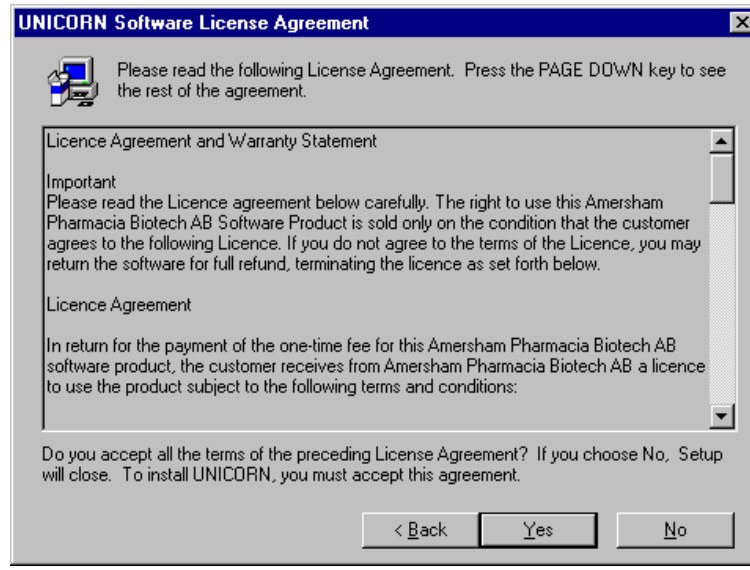

UNICORN Software License Agreement
Please read the following License Agreement. Press the PAGE DOWN key to see the rest of the agreement.
Licence Agreement and Warranty Statement
Important
Please read the Licence agreement below carefully. The right to use this Amersham Pharmacia Biotech AB Software Product is sold only on the condition that the customer agrees to the following Licence. If you do not agree to the terms of the Licence, you may return the software for full refund, terminating the licence as set forth below.
Licence Agreement
In return for the payment of the one-time fee for this Amersham Pharmacia Biotech AB software product, the customer receives from Amersham Pharmacia Biotech AB a licence to use the product subject to the following terms and conditions:
Do you accept all the terms of the preceding License Agreement? If you choose No, Setup will close. To install UNICORN, you must accept this agreement.
< Back
Yes
No

Figure 13-2. Unicorn Software License Agreement dialogue.

7. Enter your name, company, and product serial number for the software. Click on the **Next** button to continue.

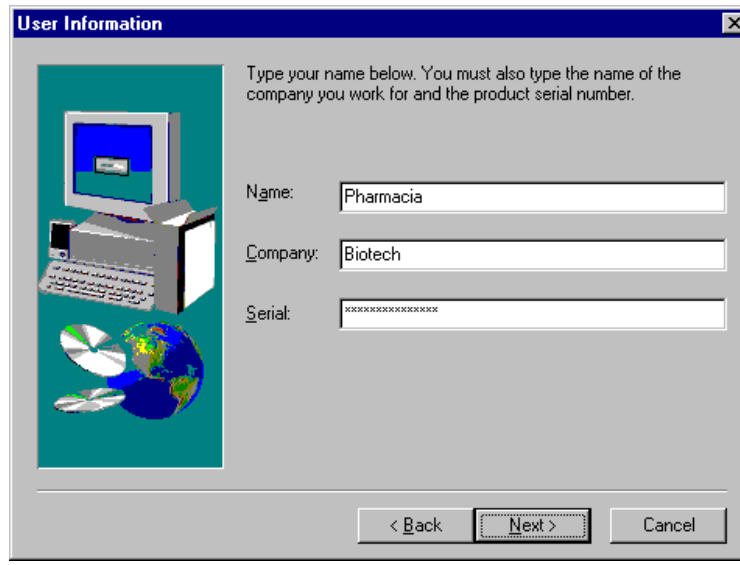


Figure 13-3. User information dialogue.

8. Select the components to install. The components **User information** and **Strategy and template files** contain sub-components. These sub-components can be accessed by clicking on the **Change** button. When all selections are made, click on the **Next** button to continue.

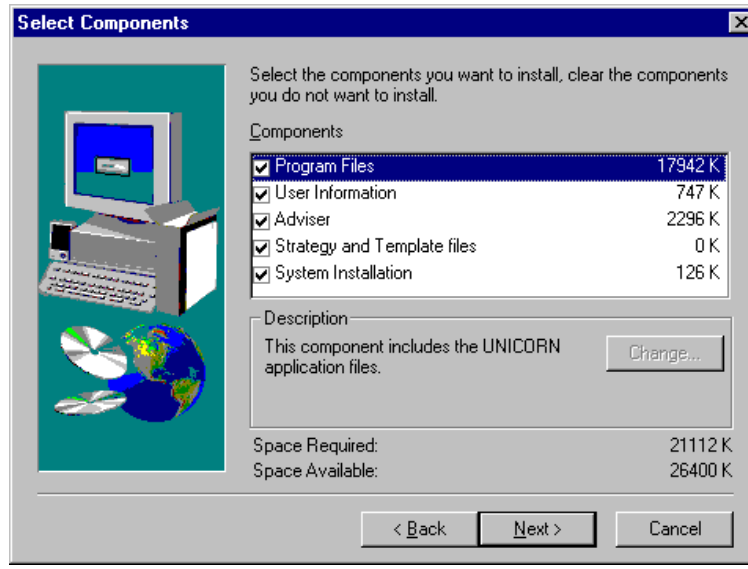

Select Components
Select the components you want to install, clear the components you do not want to install.
Components
Program Files 17942 K
User Information 747 K
Adviser 2296 K
Strategy and Template files 0 K
System Installation 126 K
Description
This component includes the UNICORN application files.
Change...
Space Required: 21112 K
Space Available: 26400 K
< Back
Next >
Cancel

Figure 13-4. Select Components dialogue.

Stand-alone installation

- Check all components

Network installation

- **Program Files** must be checked on all stations, both local and remote.
- **User Information** contains global files for the UNICORN software, procedures, BufferPrep recipes, report formats, column files and the users file. Click on the **Change** button to select sub-components to install. The users file should be checked for the first installation only. If the users file is checked during later installation on other computers in the network, any users already defined will be deleted and only the default user will be available.
- **Adviser** should be checked on all stations, both local and remote.

- **Strategy and Template files** should be checked for each new strategy to be installed (usually when a new system is installed on a local computer). Each strategy need only be installed once since they are stored on the server.
- **System** should be checked on all stations where a system is connected, but not on demo or remote stations.

If global procedures, report formats, BufferPrep recipes, columns or a users file already exist, you will be asked if you want to replace the corresponding files. Normally you should answer **No**. If you answer **Yes**, all existing global procedures, report formats, BufferPrep recipes, columns and the users file will be deleted and replaced by the defaults.

9. Select the disk drive where the program is to be installed and click on the **Next** button to continue. This should be a physical disk drive (usually c:) on the computer where you are installing UNICORN, not a network disk drive.

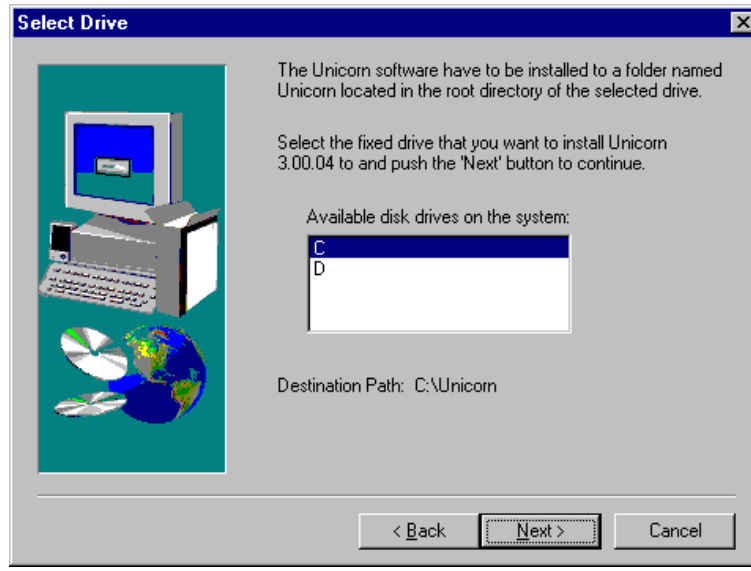

Select Drive
The Unicorn software have to be installed to a folder named Unicorn located in the root directory of the selected drive.
Select the fixed drive that you want to install Unicorn 3.00.04 to and push the 'Next' button to continue.
Available disk drives on the system:
C
D
Destination Path: C:\Unicorn
< Back
Next >
Cancel

Figure 13-5. Select Drive dialogue.

10. Choose whether the setup type is stand-alone or network.

    For a stand-alone installation the network options settings are ignored. A stand-alone installation can be either a local station or a demo station. If you want to install a demo station, make sure that **Demo system** is checked.

A network installation can be either a local station or a remote-only system. To get a remote-only system, i.e. a computer to which no systems are physically connected, check **Remote-only system**. For a network installation you will also have to select a server disk where the server files are to be located.

When you perform a network installation, the necessary parts of UNICORN software will be copied automatically to the network server disk.

Note: If you perform a stand-alone installation and later want to connect the system to a network, you must re-install UNICORN software with the appropriate settings.

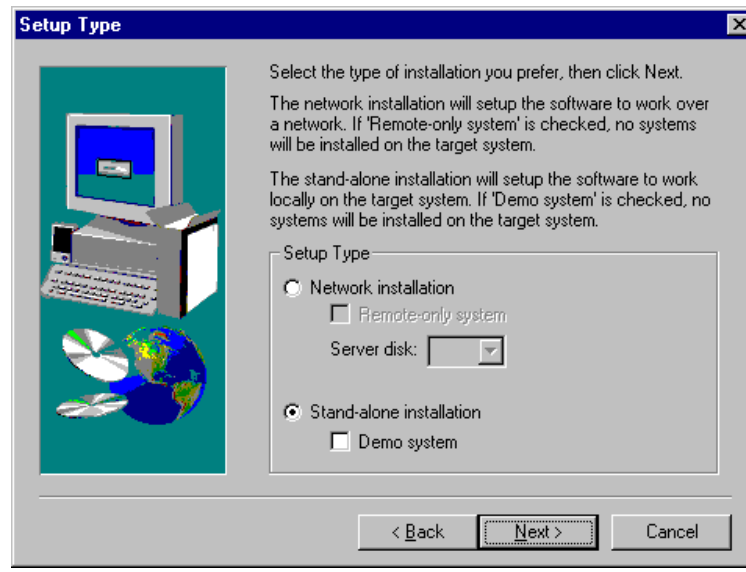

Setup Type
Select the type of installation you prefer, then click Next.
The network installation will setup the software to work over a network. If 'Remote-only system' is checked, no systems will be installed on the target system.
The stand-alone installation will setup the software to work locally on the target system. If 'Demo system' is checked, no systems will be installed on the target system.
Setup Type
Network installation
Remote-only system
Server disk:
Stand-alone installation
Demo system
< Back
Next >
Cancel

Figure 13-6. Setup Type dialogue.

11. Enter the minimum number of characters required for passwords (Valid numbers of password characters are 3 -15). Alternatively, if you do not require password protection, check **No password required**. With this setting, users can be defined with or without passwords.

Note: In a network installation, make sure that you enter the same password settings on each station in the network.

Choose the number of system control windows that should be available in the installation (maximum 4).

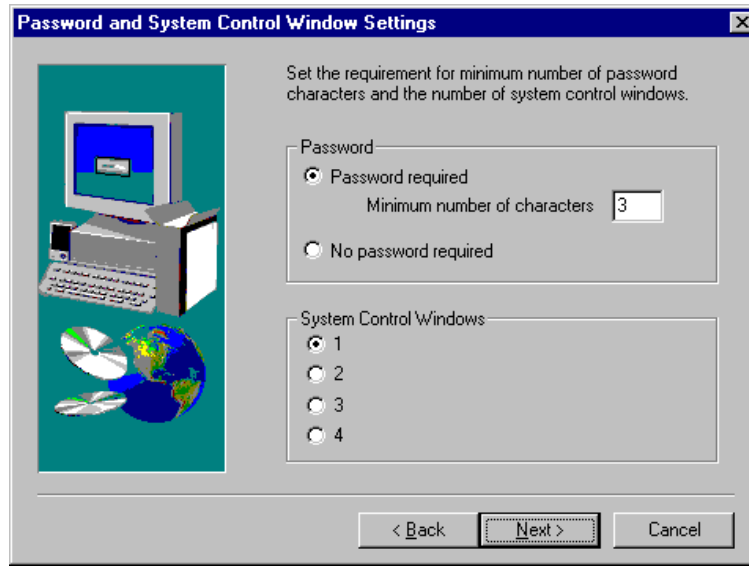


Figure 13-7. Password and System Control Window Settings dialogue.

12. Select the program folder in which you want the UNICORN icon to be placed. You can either create a new folder or select one that already exists. The folder will be placed in the programs menu under the Start button menu.

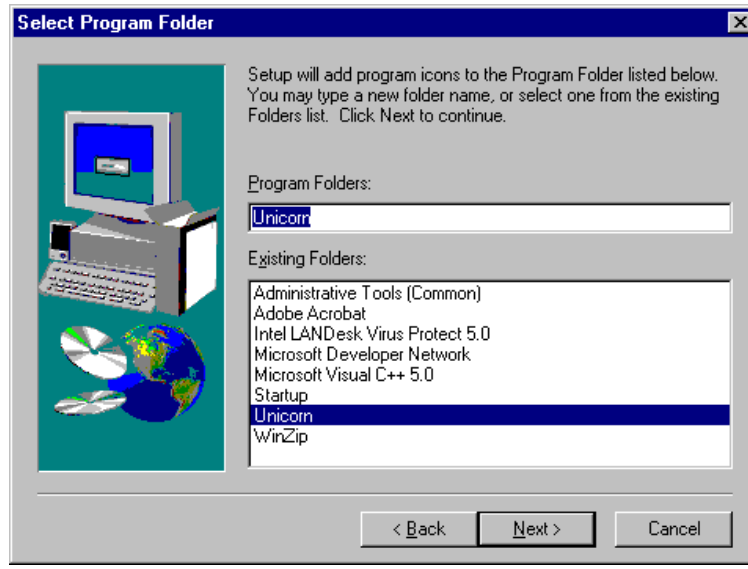

Select Program Folder
Setup will add program icons to the Program Folder listed below. You may type a new folder name, or select one from the existing Folders list. Click Next to continue.
Program Folders:
Unicorn
Existing Folders:
Administrative Tools (Common)
Adobe Acrobat
Intel LANDesk Virus Protect 5.0
Microsoft Developer Network
Microsoft Visual C++ 5.0
Startup
Unicorn
WinZip
< Back
Next >
Cancel

Figure 13-8. Select program Folder

13. At this point, the setup program is ready to start copy the files. A dialogue will display all the selections that have been made. If the settings are correct click on the **Next** button to start copying the files. If you want to make any changes you can click on the **Back** button.

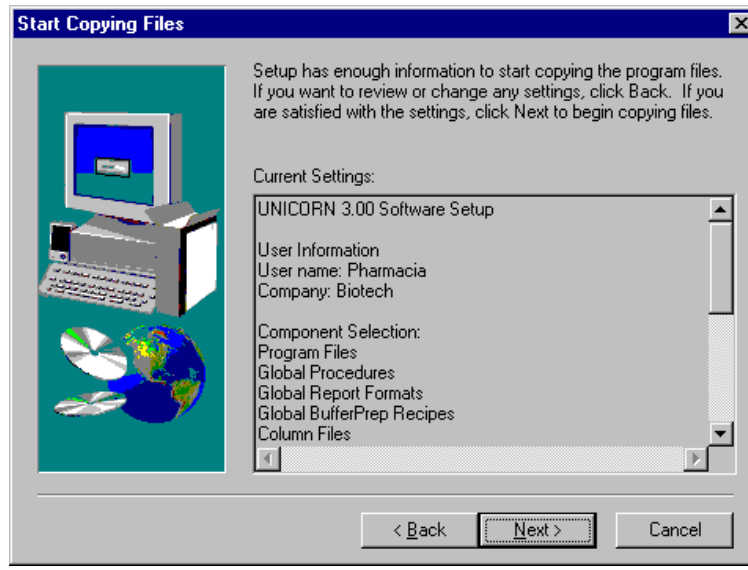

Start Copying Files
Setup has enough information to start copying the program files. If you want to review or change any settings, click Back. If you are satisfied with the settings, click Next to begin copying files.
Current Settings:
UNICORN 3.00 Software Setup
User Information
User name: Pharmacia
Company: Biotech
Component Selection:
Program Files
Global Procedures
Global Report Formats
Global BufferPrep Recipes
Column Files
< Back
Next >
Cancel

Figure 13-9. Start Copying Files dialogue.

14. If you have chosen to install systems, the system installation dialogue is displayed after the file copy process. Select a system and click on the **Change** button to set up the system. When all systems that are connected to the station have been defined click on the **Next** button to continue.

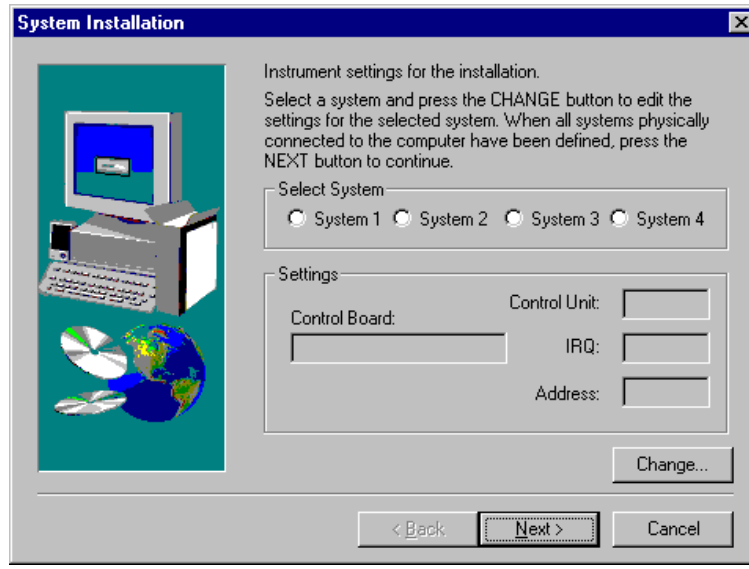


Figure 13-10. System Installation dialogue.

When you click on the **Change** button, the **System Setup** dialogue is displayed.

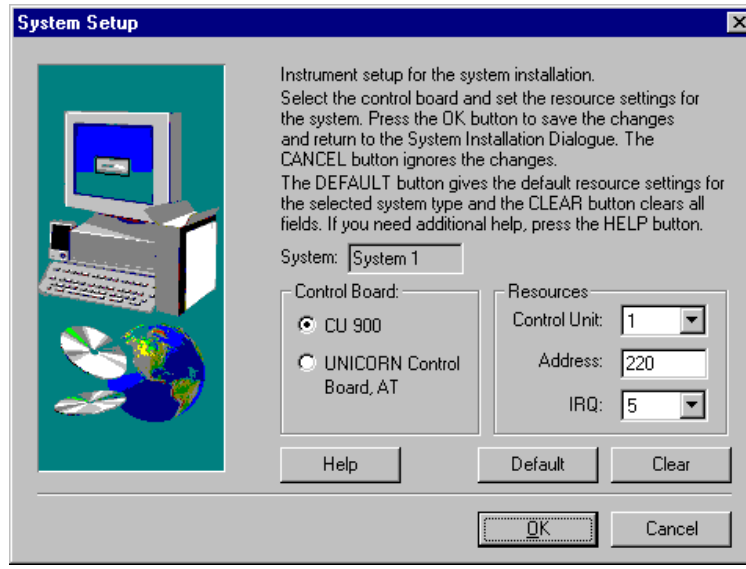

System Setup
Instrument setup for the system installation.
Select the control board and set the resource settings for the system. Press the OK button to save the changes and return to the System Installation Dialogue. The CANCEL button ignores the changes.
The DEFAULT button gives the default resource settings for the selected system type and the CLEAR button clears all fields. If you need additional help, press the HELP button.
System:
System 1
Control Board:
CU 900
UNICORN Control Board, AT
Resources
Control Unit:
1
Address:
220
IRQ:
5
Help
Default
Clear
OK
Cancel

Figure 13-11. System Setup dialogue.

Select the type of control board that the system being installed is using, **CU 900** or **UNICORN control board, AT**. For each system you will have to set the correct settings for the control unit, interrupt and address. Refer to chapter 13.3 for the correct settings. The **Default** button gives the default resource settings for the selected system type. If you want to remove a system, click on the **Clear** button to clear all fields. All fields must contain a value before you can continue. Click on **OK** to save the changes or **Cancel** to abort the dialogue. In both cases you are returned to the **System Installation** dialogue. Install additional systems until you have installed all systems physically connected to the computer (the number of systems is not related to the number of system control windows installed in step 12). If you installed more than one system, make a note of which system is connected to which control unit. This information is useful to have when you set up the system table or if you must in the future reinstall UNICORN.

Note: If you want to define systems later or change the settings for an already defined system, run the setup program once again with only the **System Installation** option checked in the **Component Selection** dialogue.

15. If you have chosen to install strategy and/or template files, the **Strategy & Template Installation** dialogue is displayed. If the correct strategy is visible in the list, mark that strategy and click on the **Install** button. If you have the strategy/template files on a diskette or another source, click the **Have Disk** button to locate the strategy/template files.

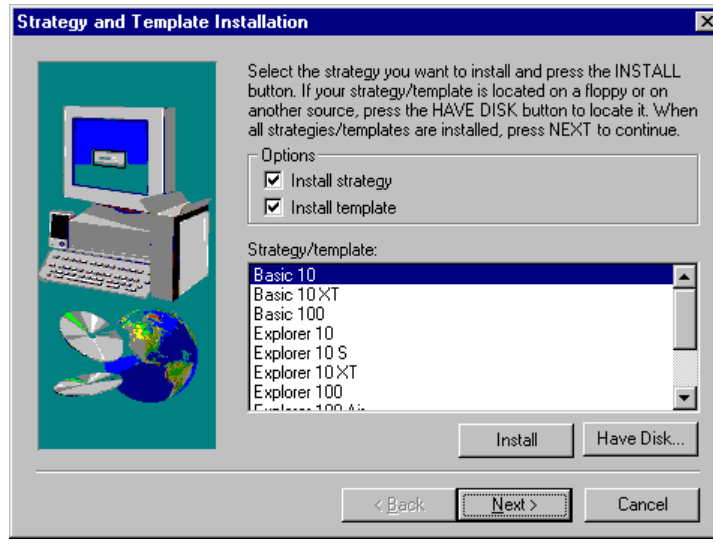

Strategy and Template Installation
Select the strategy you want to install and press the INSTALL button. If your strategy/template is located on a floppy or on another source, press the HAVE DISK button to locate it. When all strategies/templates are installed, press NEXT to continue.
Options
Install strategy
Install template
Strategy/template:
Basic 10
Basic 10 XT
Basic 100
Explorer 10
Explorer 10 S
Explorer 10 XT
Explorer 100
Install
Have Disk...
< Back
Next >
Cancel

Figure 13-12. Strategy & Template Installation dialogue.

If the options for both strategy and template are checked the template files are installed automatically together with the strategy. If the strategy and template files are located on different diskettes, the setup program will ask for the template disk when it is needed.

When installing strategies, you will be prompted for a name for the strategy that is being installed. You can either keep the default name or enter another name. The name for a strategy can not be more than 8 characters.

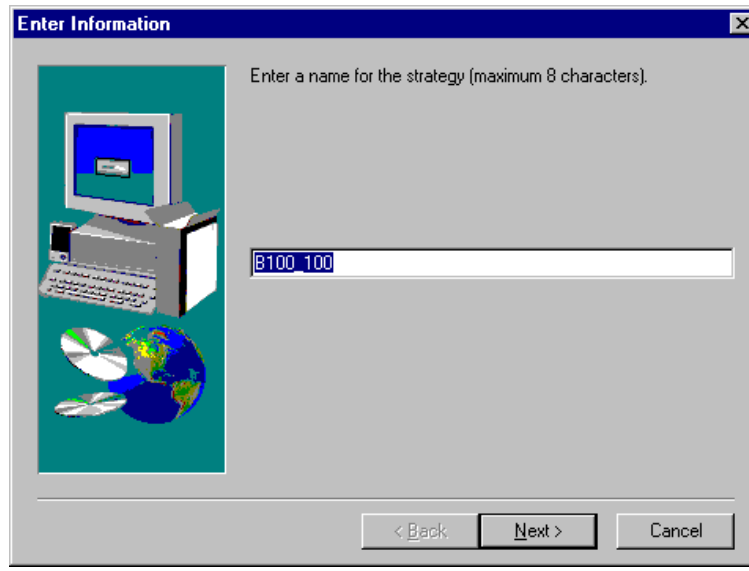


Figure 13-13. Enter Information dialogue.

If you are installing template files separately, you must enter the appropriate destination for the correct files. It is not possible to detect which template files correspond to which strategy. A list with all strategies installed on the station is displayed and you must select the strategy for which you want to install the template files.

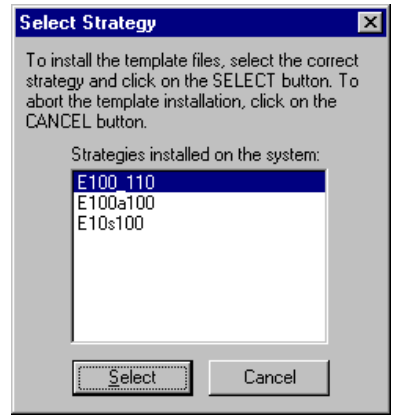

Select Strategy
To install the template files, select the correct strategy and click on the SELECT button. To abort the template installation, click on the CANCEL button.
Strategies installed on the system:
E100_110
E100a100
E10s100
Select
Cancel

Figure 13-14. Select Strategy dialogue.

If you click on the **Select** button, the template files will be installed to the folder where the selected strategy is located. The **Cancel** button aborts the template installation. In both cases you are returned to the **Strategy & Template Installation** dialogue.

Choose to install additional strategies if you have more than one system configuration. Strategies are installed independently of systems. A strategy is assigned to a system when the system is defined.

16. The **System Table Settings** dialogue is only displayed when UNICORN is installed for the first time and only if a system table is not already present. The dialogue will be shown for each system defined in the **System Setup** dialogue (see step 14 above). Enter a name that you want to use for the system. If you want to connect a strategy to the system, select a strategy in the drop-down list box where all installed strategies are visible. When all selections have been made, click on the **OK** button to save the system in the system table. If you click the **Cancel** button, the system will not be written to the system table and you have to enter it yourself from within the UNICORN software (in the Main menu **Administration: System setup**).

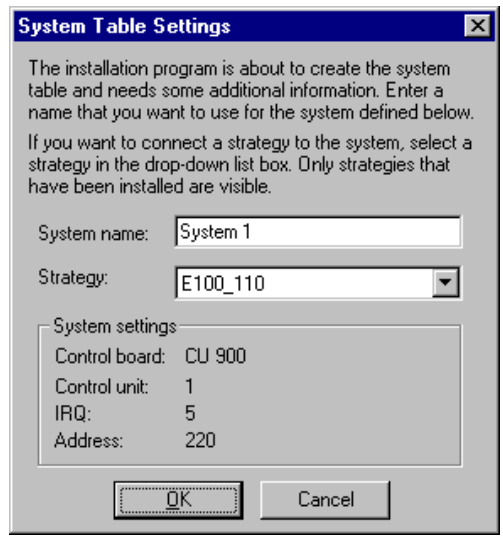

System Table Settings
The installation program is about to create the system table and needs some additional information. Enter a name that you want to use for the system defined below.
If you want to connect a strategy to the system, select a strategy in the drop-down list box. Only strategies that have been installed are visible.
System name:
System 1
Strategy:
E100_110
System settings
Control board:
CU 900
Control unit:
1
IRQ:
5
Address:
220
OK
Cancel

Figure 13-15. System Table Strategy dialogue.

17. After the installation is complete the computer must be restarted for the installed software to be properly configured. Click on **Finish** to exit the **UNICORN Setup** dialogue and restart the computer.

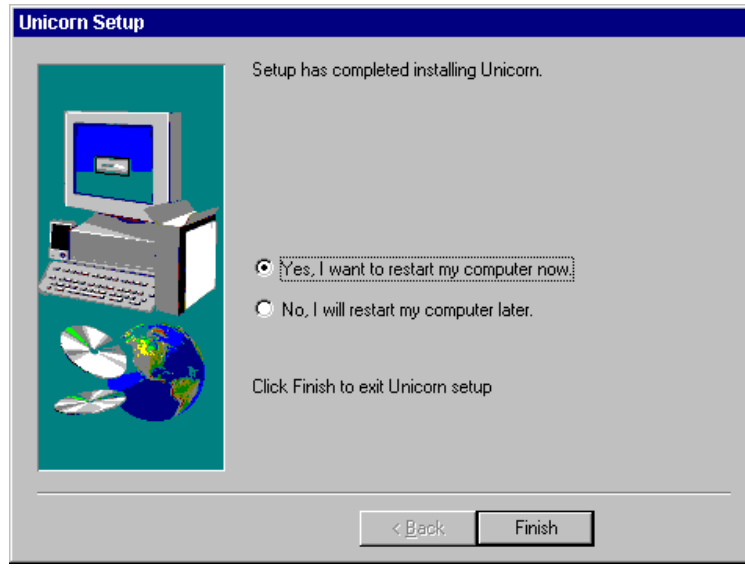

Unicorn Setup
Setup has completed installing Unicorn.
Yes, I want to restart my computer now.
No, I will restart my computer later.
Click Finish to exit Unicorn setup
< Back
Finish

Figure 13-16. UNICORN Setup dialogue.

13.5.2 Installing selected software components after the initial installation

If your UNICORN installation should be damaged, for example due to accidental file deletion or hard disk failure, or if you want to install additional systems, strategies or templates, you can use the installation program to re-install selected parts of the software. The installation program detects the presence of existing UNICORN files, and suggests the components to install. Check some or all of **Program files**, **User information**, **Adviser**, **Strategy & template files** and **System installation** according to which part(s) you wish to install. The appropriate parts will be copied into the existing folder structure.

Note: For network installations, remember to log on to the network before installing any UNICORN software components.

Program files

Before re-installing the program files, be sure that the UNICORN software is not running. Check the **Program files** option in the **Components** list to re-install UNICORN. This will not affect any existing method or result files in the system. The program is always installed locally, even in network installations.

User information

User information contains several sub-components, global procedures, global report formats, global BufferPrep recipes, column files and the users file. To re-install these global files, check each sub-component.

Note: Any changes to the files will be lost since they will be replaced with their defaults.

If, for example, the existing user definitions are damaged you can check the **Users file** to re-install the default user. Any other users defined in the system including users installed from other stations in a network installation will be deleted. However, method and result files will not be erased. You can regain access to these files by re-defining users with appropriate folder access. Users are installed on the network server in a network installation.

System installation

Check the **System installation** option if you are installing a new or an additional system on a stand-alone computer or a local station in a network, or if you want to change the settings for an existing system. Systems are not installed on demo or remote-only systems.

Strategy files

Check the **Strategy files** option to re-install system strategies or to install additional strategies. Accept the suggested name or enter a new name for the strategy. This will not affect any existing method or result files in the system. Strategies are installed on the network server in a network installation.

Template files

Check the **Template files** option to re-install template files or to install template files for a new strategy. Normally, templates are installed together with strategies. If you are not installing a strategy at the same time, a dialogue will be shown displaying all strategies that are installed on the system. You must select the strategy to which the method template files correspond. Templates are installed on the network server in a network installation.

14 Administration

There are three main aspects of administration of a UNICORN installation:

- System administration
- Access level and user administration
- Network administration where appropriate.

System administration concerns maintenance of software aspects of the installation, including definition of connected systems and monitoring of system usage (audit trails). These activities are described in the present chapter. System administration duties may also include routine monitor calibration (Section 6.6).

Access level and user administration concerns authorisation of access to the system, and should (at least in larger installations) be the responsibility of one person or a small group. These activities are described in the present chapter.

In a network installation, maintenance of the network functions will normally be carried out by the computer staff responsible for the company's network. Aspects of network administration relevant to UNICORN are considered in Chapter 12.

After installation, the following operations should be performed in UNICORN by the administrator before other users can use the program:

1. Set up system definitions for the chromatography systems (Section 14.1).

2. Define access levels for the installation (Section 14.2).

3. Define new users with home folders and access profiles (Section 14.3).

Note: These operations can be performed on any station in a network installation. It is however important that the administrator is logged on to the network on the station being used so that the changes will apply globally throughout the network.

14.1 System definitions

System definitions set up the chromatography systems which are connected directly to the local computer in UNICORN installation. This must be done for each new system installed. In a network installation, these definitions must be set up for each local computer in the network, but the actual set-up operations can be performed on any computer. Rights of access to system are controlled at the user administration level (Sections 14.2 and 14.3).

To manage system definitions, choose **Administration:System setup** in the Main menu. To use this menu command, you must have **Audit trail/ System setup** authorisation (see above).

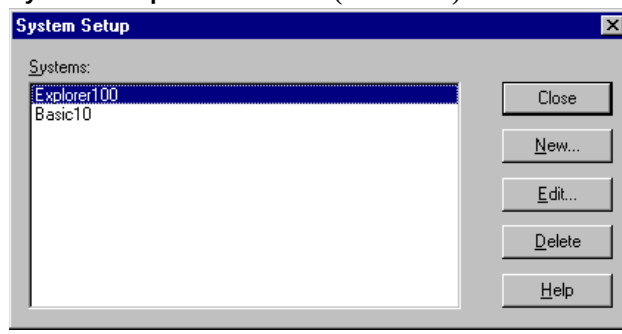


Figure 14-1. System setup dialogue.

14.1.1 Defining new systems

To define a new system, click on **New** in the **System setup** dialogue. The **New system** dialogue is displayed.

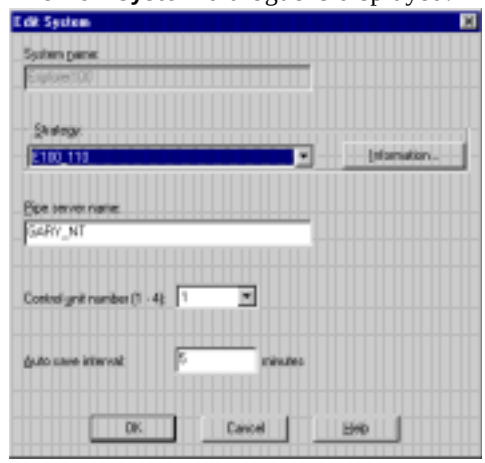


Figure 14-2. New system dialogue.

1. Enter the system name in the **System name** field. The system name can be set only when defining a new connection, and cannot later be edited since users' access rights are linked directly to the system name. Names can be up to 30 characters long.

2. Select a strategy for the system from the pull-down list in the **Strategy name** field. Available strategies are determined when UNICORN is installed (see Section 13.4). If you have several strategies installed, make sure that the selected strategy is appropriate for the chromatography system being defined. Click on **Information** to display information about the selected strategy.

3. The **Pipe server name** field is the same as the Windows NT Computer Name. Leave this value unchanged.

4. Select the control unit number (1-4) in **Control unit number**. This is the physical connection number for the chromatography system on the local computer (see Chapter 13).

5. Enter a value in the **Autosave interval** field if you want UNICORN to save a copy of the result file at pre-set intervals during a run. This minimises loss of data in the event of a computer failure. The recommended interval for most systems is 5 minutes. A shorter interval may slow down the user interface response. The control functions in UNICORN performance will however not be impaired.

Note: Normally, you should define systems before defining users. If you add system definitions after you have defined users, remember to grant access to the new systems to the appropriate users (see Section 14.3).

14.1.2 Editing system definitions

To edit an existing system, select the system in the **System setup** dialogue and click on **Edit**. Alter the parameters as appropriate (see Section 14.1.1 for a description of the dialogue contents).

Remember that if you change the system strategy the arrangement of tubing, pumps, columns etc. may need to be changed on the chromatography system. An attempt to control a system using the wrong strategy may cause malfunction and damage to the chromatography system.

14.1.3 Deleting system definitions

To delete a system definition, select the system(s) in the **System setup** dialogue and click on **Delete**. A system definition can only be deleted if the system is idle and no users are connected to the system.

14.2 Access levels

Access to UNICORN software is controlled by username and password authorisation (see Sections 14.3 and 3.1). Within the program, each authorised user is assigned an access level within the system which determines which functions the user can perform:

14.2.1 Defining access levels

Up to 10 different access levels can be defined. Initially, all levels are the same with access to all functions.

To edit an access level, select **Administration:Access levels** from the Main menu and select the level you wish to edit. To use this menu command, you must have **User setup/Levels** authorisation (see below). The levels are named **Level 1 - Level 10** by default: to change the name of a level, enter a new name click on the **Rename** button. Check the items to which users at this level are to have access, and click on **OK**. If you change the definition of a level to which users are already assigned, the changes will apply to all users at this level.

At least one access level must have **User setup/Levels** authorisation. UNICORN will not allow you to remove this authorisation from all levels.

The authorisation items are:

- **Method Editor**
  Required for using the Method editor for creating and editing methods (Chapters 4 and 5).
- **Evaluation**
  Required for using the evaluation module for processing result data (Chapters 9 and 10).
- **User setup/Levels**
  Required for defining and changing access levels and user.

  IMPORTANT!
  We recommend that only one user in an installation or network is assigned this access.

- **Audit trail/System setup**
  Required for examining the audit trail and for defining connected systems (Section 14.4).

  IMPORTANT!
  We recommend that only one user in an installation or network is assigned this access.

- **Delete, move - Home only**
  Required for deleting and moving files and folders within the user's home folder (Sections 3.2.5 and 3.2.6). Does not authorise these operations on other folders.
- **Delete, move**
  Required for deleting and moving files and folders outside the user's home folder (Sections 3.2.5 and 3.2.6). Also authorises these operations within the home folder.
- **Copy file(s)**
  Required for copying files (Section 3.2.5). The user must have access to both the source and target folders for moving or copying between folders.
- **Confirm**
  Required for authorised confirmation of answers to start protocol questions (Section 5.6.3).
- **Unlock locked system**
  Permits a user to unlock locked systems by providing the user's own logon passwords (locked systems can normally only be unlocked using the locking password, see Section 6.5.3). We recommend that this authorisation is restricted to a few users in an installation. The user who locks a system does not require this authorisation to unlock the same system.
- **Run methods**
  Required for starting methods (Section 6.1).
- **Manual interaction**
  Required for issuing manual commands in System control (Section 6.3).
- **Pause**
  Required for pausing a running process with the PAUSE button in System control (Section 6.3.1). The PAUSE instruction in methods does not require explicit authorisation.
- **Calibrate/Tune**
  Required for using the Calibrate and Tune commands in System control (Section 6.6 and Appendix D).
- **System settings**
  Required for changing system settings with the Settings command in System control (Chapter 15). Any user may view the system settings, but this authorisation is required to make changes to the settings.
- **Edit MethodQueue**
  Required for using the MethodQueue editor (Sections 8.1 and 8.2).

- **Run MethodQueue**
  Required for running MethodQueues (Section 8.3).
- **Edit global list(s)**
  Required for saving a method as a method template (Section 5.7.2), an evaluation procedure (Section 10.3), a report format, a column in the Column list or BufferPrep recipe (also Quantitation tables and Mol Size tables if the Analysis module is installed) as globally available. It is also required for deleting method templates, global procedures, global report formats, global columns or global BufferPrep recipes (also global Quantitation tables and global Mol Size tables). We recommend that this authorisation is restricted to only one user in an installation.
- **Quit program**
  Required for ending a UNICORN session with the **File:Quit program** command in the Main menu.

14.2.2 Access level examples

Below are some examples illustrating the way access levels might be used in a multi-user installation.

System administrator

The administrator has special responsibility for maintaining user, system and audit information and for file management in the PC (folder structure, backup routines, etc.). The administrator may not edit or run methods or MethodQueues, issue manual instructions, calibrate monitors or change the system configuration.

IMPORTANT!
We recommend that only one user in an installation or network is assigned system administrator rights. If several users can change user definitions and system connections, confusion can rapidly follow.

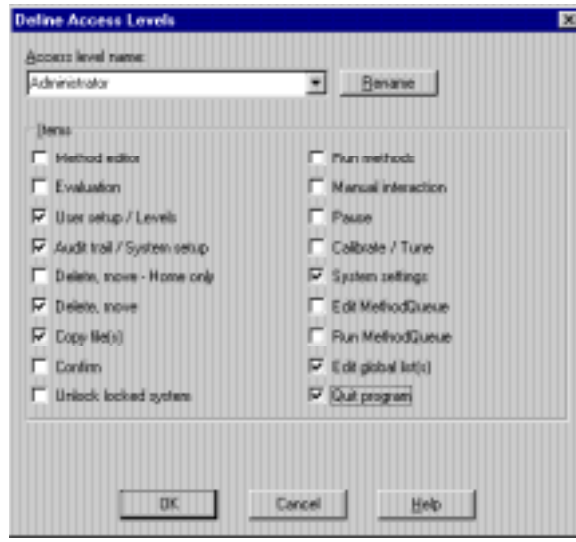


Figure 14-3. Suggested authorisation profile for the system administrator.

Development staff

Developers need to be able to edit and run methods and MethodQueues, issue manual instructions, configure system parameters, calibrate monitors and evaluate data. They may also copy, move or delete files.

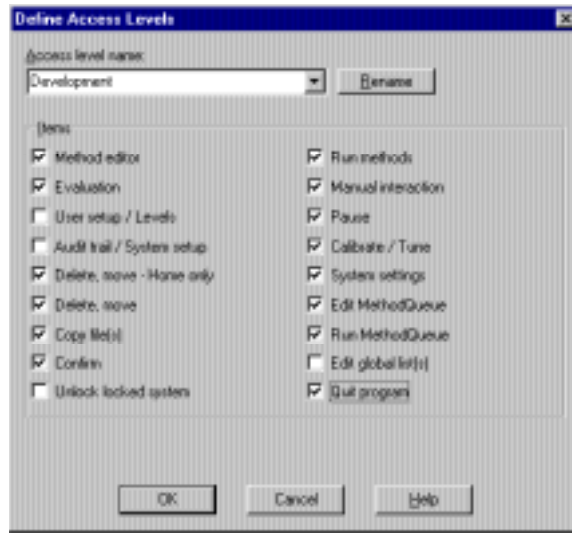


Figure 14-4. Suggested authorisation profile for development staff.

Process supervisors

Supervisors may pause a method and issue manual instructions as well as start methods and MethodQueues. Supervisors are also allowed to calibrate monitors, configure system parameters, unlock running processes, evaluate run data and quit UNICORN.

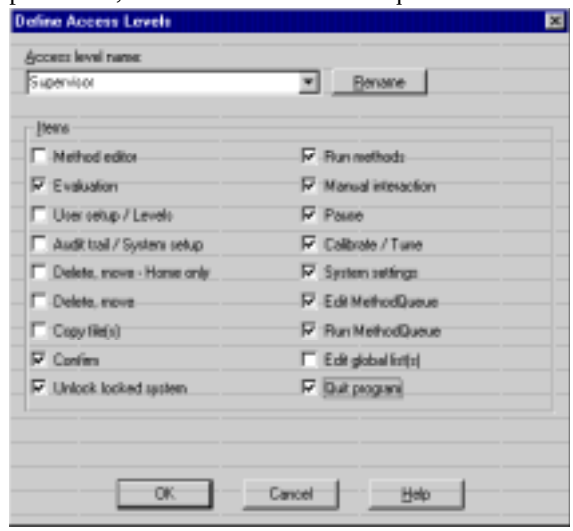


Figure 14-5. Suggested authorisation profile for process supervisors.

Process operators

Process operators are allowed to run and pause methods and MethodQueues but may not perform any other operations.

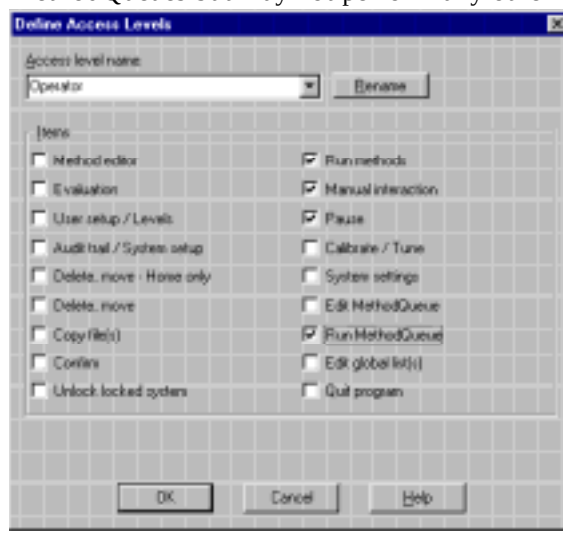


Figure 14-6. Suggested authorisation profile for process operators.

14.3 User administration

All UNICORN users are identified by a username and password. A new installation is provided with a default user (username:**default**, password:**default**). This user provides unrestricted access to all UNICORN functions.

IMPORTANT!
As part of the installation procedure, new users should be created with passwords and restricted access rights as required. The default user should be deleted or redefined to prevent unauthorised access to the system.

Maintenance of user authorisation information is the responsibility of the system administrator. In a newly installed system, log on as user **default**.

To define, edit or delete users, choose **Administration:User setup** in the Main menu. To use this menu command, you must have authorisation (see above). All user administration is performed from this dialogue.

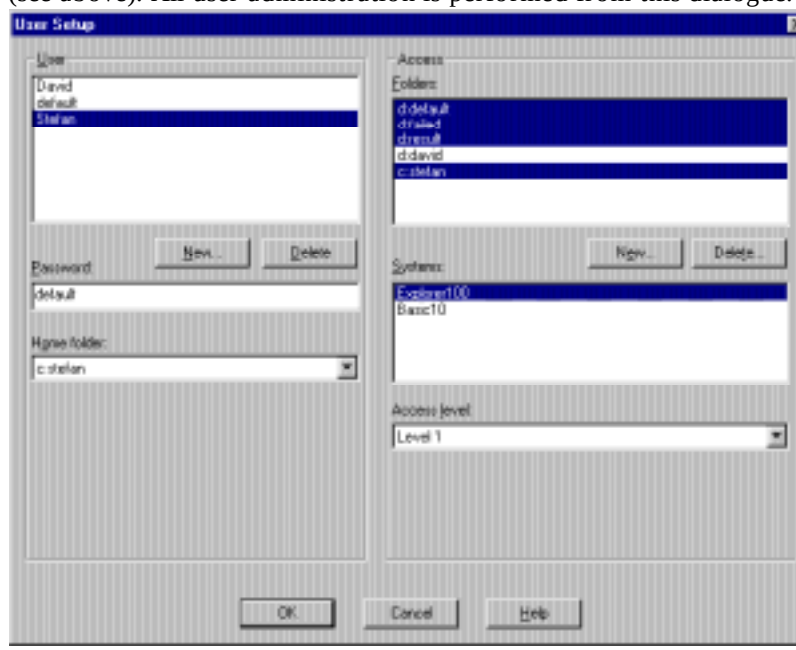


Figure 14-7. The User set-up dialogue.

14.3.1 Defining new users

1. To define a new user, click on **New** in the **User** field and enter the username in the displayed dialogue.



Figure 14-8. dialogue for defining a new user.

2. To create a new home folder for this user, check the **Create home folder** box and enter the folder name and drive in the appropriate fields. If you choose to create a home folder on a network drive, make sure that the drive is always accessible. Click on **OK** to create the user and return to the **User setup** dialogue.

Note: A home folder can always be created on a network drive even if UNICORN is not installed for network control. The computer only needs to be connected and logged on to the local network.

In general, each user should have a separate home folder.

IMPORTANT!
In a network installation, always create home folders on a network drive which is accessible from all computers. If you create a home folder on the C: (local) drive, it will only be accessible from the computer on which it was created.

3. Enter the user's password in the **Password** field. The minimum number of characters in the password is defined when UNICORN is installed (see Section 13.3). The program may also be installed without password protection. The case of letters in passwords is significant.

IMPORTANT!
Leaving the password as default can constitute a serious security risk.

All user passwords are visible in the **User setup** dialogue. For security reasons, make sure that access to this function is restricted.

4. Select a home folder from the **Home folder** drop-down list. You may choose any folder from this list, even if you created a home folder in the **New user** dialogue.

5. In the **Access** field, select other folders to which the user will have access from the folders list. Up to 20 folders can be set up to be shared. Selecting a folder here will give the user access to all files and folders therein. Folders that are not selected in this list will not be visible in the methods or results panel of the Main menu.

Note: All users should be given access to the Failed folder on each local station in a network installation. This will ensure that users can access results saved in the Failed folder in the event of a network communication error.

6. Select the system(s) to which the user will have access from the System(s) list and the access level of the user from the **Access level** drop-down list.

You can continue to define new users as long as the **User setup** dialogue is open. Click on **OK** to close the dialogue. If you close the dialogue by clicking on **Cancel**, all changes made since you opened the dialogue will be lost.

14.3.2 Changing user passwords

Every user can change his or her own password with the **Administration:Change Password** command in the Main menu. Enter the old password and the new password in the appropriate fields. Passwords are not displayed explicitly in this dialogue. The password will not be changed if either the old password is incorrect or the two copies of the new password differ from each other.

In addition, a user with **User setup/Levels** access can change the password for any user. To change a password for another user, open **Administration:User setup**, select the user and enter a new password in the **Password** field (see Section 14.3.1).

Change passwords regularly and avoid obvious passwords like "secret" and "open_sesame" for maximum security.

If you forget the password for the only user with **User setup/Levels access**, you must re-install the default user as described in Section 13.4.2.

14.3.3 Viewing and changing user definitions

To view the setup for any user, click on the user name in the **User setup** dialogue. To change the user definitions, make changes in the dialogue fields as appropriate and click on **OK**.

14.3.4 Deleting users

To delete a user, select the user from the username list and click on **Delete**. You may delete all users except the last user with **User setup/ Levels** access. This ensures that at least one user has the right to perform administration functions.

Deleting a user does not affect the user's home folder or method and result files.

14.3.5 Defining new home folders

To define an existing folder as a home folder for a user, select the folder in the **Home folder** field in the **User setup** dialogue. Any folder may be used as a home folder. As a recommendation in network installations, place the home folder on a drive which is addressed by the same drive letter from all computers in the network.

To create a new home folder at the same time as you create a user, check the **Create home folder** option in the **New user** dialogue and enter the folder name and drive in the appropriate fields.

To create new folders under a home folder, use the **New folder** command in the Main menu (see Section 3.2). This operation does not require special authorisation.

14.3.6 Deleting home folders

To delete home folders, click on **Delete** in the **Access** field of the **User setup** dialogue. Select a folder to delete in the **Delete Folder** dialogue that is displayed and click on **OK**.

IMPORTANT!
It does not matter which folders are marked in the folders list in the **User setup** dialogue when you click on **Delete**. This list shows the folders to which the currently marked user has access, not the folders that will be deleted.

All methods, result files and folders within a selected folder will be deleted when the selected folder is deleted.

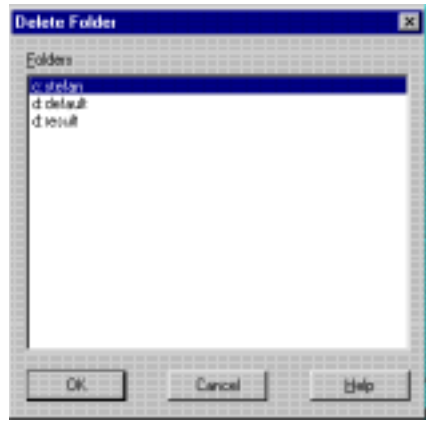


Figure 14-9. Delete Folder dialogue.

You cannot delete:

- a home folder to which a user is assigned. To delete such a folder, you must first either delete the user or change the home folder assignment for this user. Select the user in the **User setup** dialogue and assign a different home folder.
- a folder to which several users share access. To delete such a folder, remove the access rights from each user first.

14.4 Audit trails

The audit trail, accessed under **Administration:Audit Trail** in the Main menu, provides a full record of UNICORN usage and system activity for the system administrator. The audit trail may be viewed in global mode (all systems in the installation) or system mode (one chosen system).

14.4.1 Examining audit trails

In the audit trail window, select whether you wish to view the **Global** or **System** trail.

Global audit trails

Check the items you want to display in the **Global** field. All items are recorded in the audit trail: the check boxes in the **Global** field only control which items are displayed. Global audit trail files are saved on the server disk in a network installation, and a network connection is required to examine global audit trails.

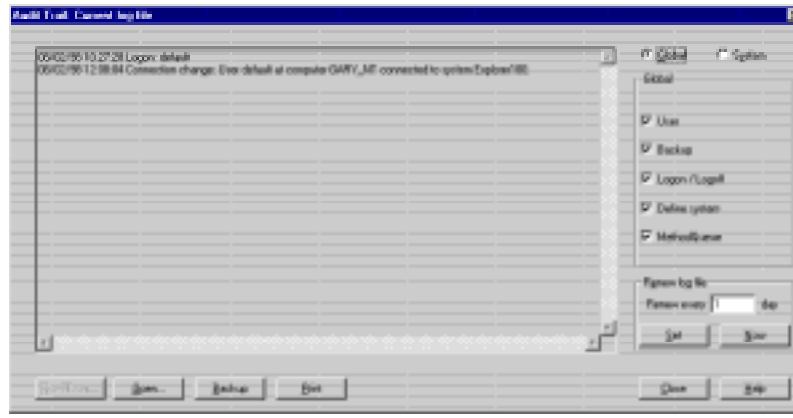


Figure 14-10. Global audit trails.

User Displays all user creation, deletion and re-definition operations.

Backup Displays backup operations for global audit trail files.

Log on/off Displays all logon and logoff attempts with the name of the user logging on or off, including failed logon attempts.

Define system Displays all system definition, deletion and redefinition events.

MethodQueue Displays MethodQueue start operations.

System audit trails

Select the system for which the audit trail is to be displayed from the drop-down list and check the items you want to view. All items are recorded in the audit trail: the check boxes in the **System** field only control which items are displayed. System audit trail files are saved on the local station to which the system is physically connected, and may be examined from the local station without logging on to the network. System audit trail files can be viewed from any computer in a network installation.

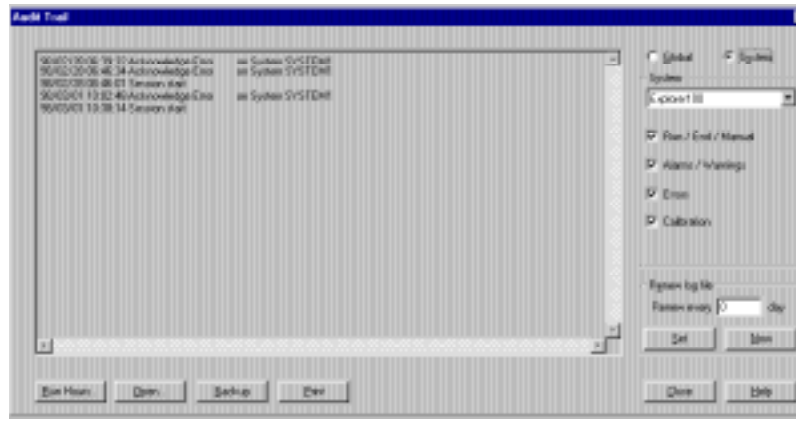


Figure 14-11. System audit trails.

Run/End/Manual Displays times for run start and completion and for manual operation.

Alarms/Warnings Displays alarms and warnings for the system.

Errors Displays system errors.

Calibration Displays monitor calibration operations.

Viewing older audit trails

When you choose **Administration:Audit trail**, the audit trail dialogue displays the current audit trail. To view an older audit trail, click on **Open** in the audit trail window and choose the file to open. The drive is automatically selected according to the type of audit trail file. If you have saved log files on diskette, these drives can be selected from the pull-down list. Files are named by date and serial number. Choose **Current log file** to return to the current audit trail after viewing older files.



Figure 14-12. Open Log File dialogue.

Printing audit trails

Click on **Print** in the audit trail dialogue menu prints the audit trail file as currently displayed in the window.

14.4.2 Renewing audit trail files

The audit trail file is renewed at regular intervals between 1 and 30 days. To set the interval, choose **Administration:Audit Trail** from the Main menu. Enter the required interval in the **Renew every** field and click on **Set**. The new setting will take effect from the time the change is made, e.g. if the setting is changed to 7 days at 10 a.m. on a Monday, the file will be renewed at 10 a.m. every Monday.

You can also start a new audit trail file at any time by clicking on **Now**. This will not affect the automatic setting, e.g. if the audit trail is set to renew at 10 a.m. every Monday, and you click on **Now** on a Friday, a new file will be started immediately and another new file will be started on the following Monday morning.

14.4.3 Backing up audit trail files

Click **Backup** in the audit trail window to make copies of audit trail files on diskette. Choose whether to copy or move the files to diskette and click on **Backup**. The **Move** alternative is recommended to save disk space. Backup operations are recorded in the audit trail.

Note: The **Backup** command simply copies the audit trail file to diskette. It does not use any Windows NT backup commands.

System run hours

In the system audit trail, click on **Run hours** to display the accumulated run time for the system (i.e. the time the system has been in manual or run mode). Run hour values show the number of hours that the system has been used for manual or method-controlled runs. The Run hours record is useful in following up expected and actual lifetimes for liquid handling components. Click on **Reset** to reset the accumulated run hours to zero. The reset time is recorded in the audit trail.

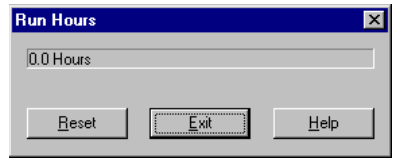


Figure 14-13. Run hours in the system audit trail.

14.5 Report Generator Wizard

UNICORN 3.00 contains a report generator Wizard for registration of errors or problems that you have detected or that occur during a run. The **Report Generator Wizard** takes you through the steps to generate your own report.

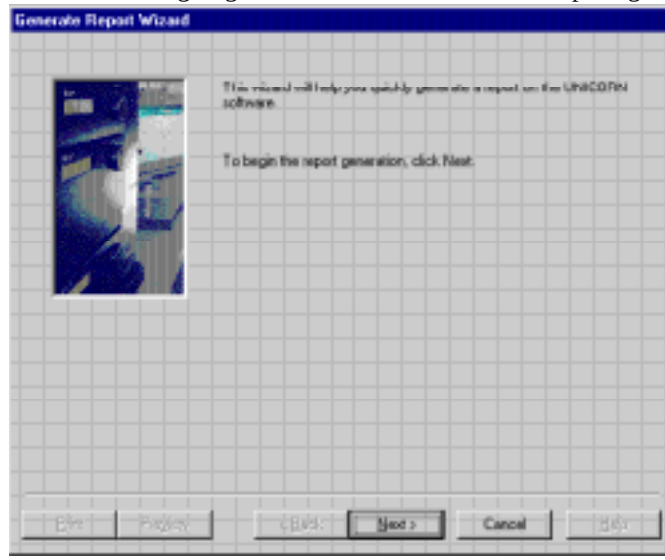
There are two ways of accessing the **Report Generator Wizard**:

- you can generate a report in the Main menu by selecting the menu command **Administartion:Create Service Log**.
- when an error message appears in System control you can activate the report generator by clicking on the **Report** button in the message dialogue.

14.5.1 Generating a report from the main menu

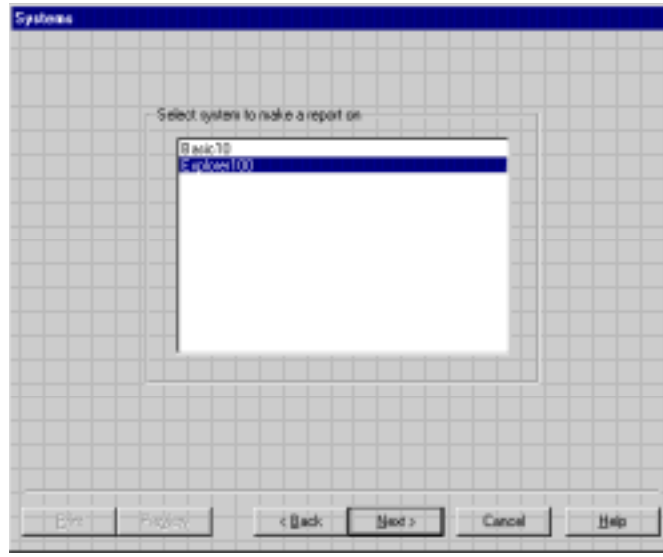
Generate Report Wizard

This first dialogue gives an introduction to the report generator.



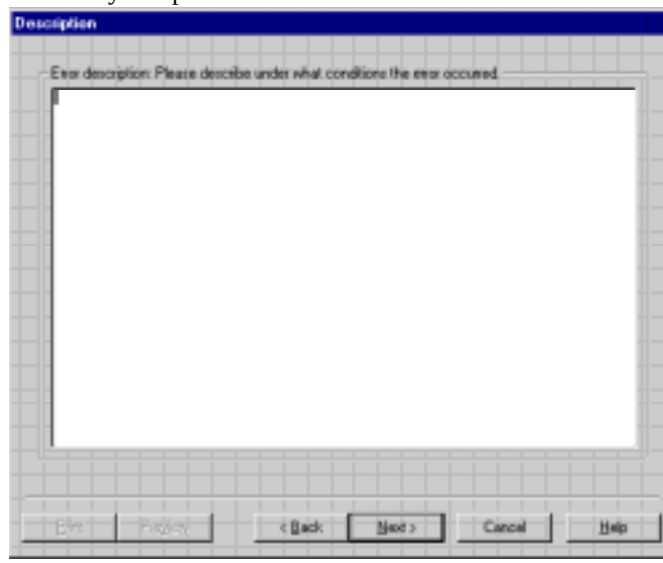
Systems

This dialogue is displayed only if you have accessed the wizard from the Main menu. This displays a list of the available systems for the logged on user. In order to proceed further, a system must be selected for which the report is to be generated.



Description

You are required to enter a short description of the problem, the circumstances under which the problem occurs, and the consequences caused by the problem.



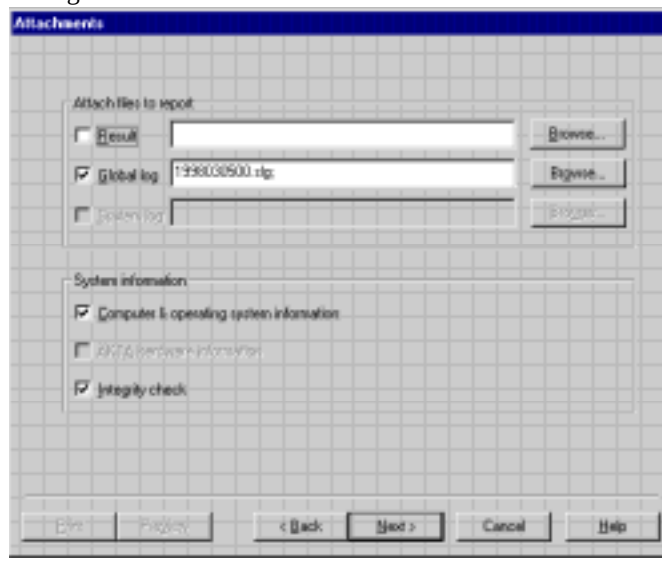
Reproducibility

You are required to give information concerning the reproducibility of the problem. If the problem is reproducible then you can describe how to bring about the problem.



Attachments

You have the option of attaching result files and/or log files to the report. Click on the **Browse** buttons and select files in the displayed dialogue.



System information can also be included in the report by checking the appropriate option boxes. By default the following options are checked:

Computer & operating system information This summarises information about the computer and operating system, e.g. type of processor, processor speed, RAM memory, hard disk capacity and printer.

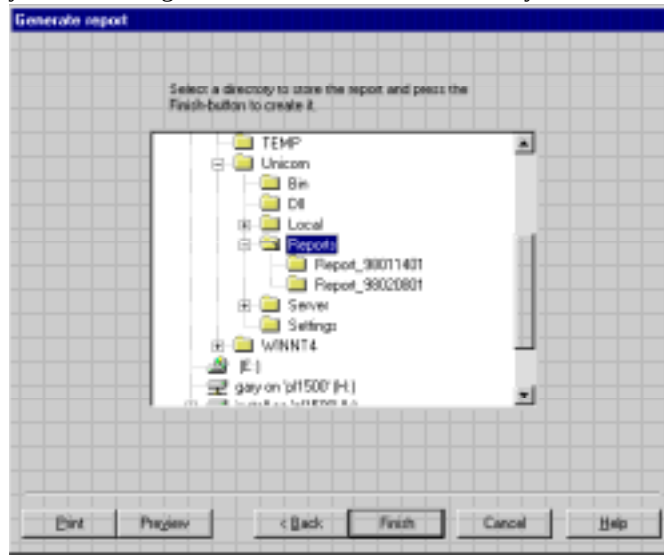
ÄKTA hardware information This summarises information about the specific ÄKTAdesign hardware, e.g the instrument and PROM version for every instrument that is connected.

Integrity check

When UNICORN is installed a checksum calculation is performed on the stationary files (*.dll and *.exe) for the system. An integrity check involves a new checksum calculation being performed for the same files in their folders and comparing the new calculated value with the checksum value obtained during installation. The results of the comparison are presented in the report and any deviations are included.

Generate Report

The Generate Report dialogue displays a tree structure of all drives that are reached from the computer performing the report generation. By default the report is saved in the folder, Unicorn\Reports although you can change this folder to another one of your choice.



You will also notice that the **Print** and **Preview** buttons are enabled in this dialogue. **Preview** opens the report in NotePad, while **Print** prints out the report without any preview.

14.5.2 Generating a report from System Control

If you activate the **Generate Report Wizard** by clicking on the **Report** button in an error message dialogue in System control, the following dialogues are displayed:

Generate Report Wizard

See description above

Description

All of the problems/errors that are currently listed in the System control error message dialogue are recorded here. Help text is then shown specific to the selected error in the error message dialogue. By selecting a different error in the dialogue, the appropriate text is shown. All problems with the help texts are included in the report.

You also have the possibility to enter a short description of the problem, the circumstances under which the problem occurs, and the consequences caused by the problem.

Reproducibility

See description above.

Attachments

See description above. One exception is that log book files can be attached instead of result files.

Generate Report

See description above.

15 System settings

Each installed system has a set of default settings, for example, to define global system settings for alarms and warnings and to select which data will be stored in result files. The default system settings are valid for all runs unless you change the settings in one of two ways:

- you assign a new value to a parameter within a method. The specific change that you make in the method is valid only until the next End instruction is given. After the End instruction the parameter returns to its default setting. Some parameter settings, however, can be set to override the global settings for the duration of the method. See Appendices B and C for settings defined in ÄKTAexplorer 100 system and other UNICORN System Controller strategies respectively.
- you assign a new value to the actual system setting. This new value is valid for all runs and remains until you again change the value or return the setting to its default value. Of course you can temporarily change the setting within a method as described in the previous point.

Changes to the default settings should be made when the system is installed. Also, certain settings may need to be adjusted if system components are changed (e.g. alarm and warning limits) or for specific run purposes (e.g. monitor and curve settings).

Assigning a new value to the actual system setting is performed in the System control module and **Configuration** access is required to make such changes (see Section 14.2).

1. To access the settings, click on the appropriate System control icon on the Windows NT taskbar and select **System:Settings**. The **Instructions** dialogue for the current system is displayed.



Figure 15-1. Instructions dialogue in System control. The illustration shows the Alarms group of settings.

2. The instructions are classified into **Instructions** groups including **Alarms**, **Special**, **Monitors** and **Curves**. To view the parameters and parameter settings for a particular group, click on the appropriate option button.

3. The instructions for the selected instruction group are listed. Beneath each instruction is listed the parameters and the current setting.

4. To change the setting for a parameter, select a parameter from the list and change the associated parameter setting. The parameter is updated in the list.

5. To restore a setting to its default setting *as defined in the system strategy*, select the setting and click on **Set Parameter to Default Value**.

6. When all required changes have been made, click on **OK**. Choosing **Cancel** will cancel all changes made since the dialogue was last opened.

IMPORTANT
Changes made to settings do not take effect until you click on **OK** to close the dialogue.

15.1 Alarms

Alarms define upper and lower alarm and warning limits for process monitor signals:

- If the signal exceeds the Alarm limits, a buzzer sounds and an alarm message is displayed, and the process is paused (i.e. method execution is suspended and all pumps are stopped). The situation must be acknowledged and corrected before the process can be restarted.
- If the signal exceeds the Warning limits, a warning message is issued without interrupting the process.

Note: Warning limits are not available for ÄKTAdesign systems.

Alarm and warning messages are displayed on all stations with a connection to the system concerned, regardless of the activity currently being performed in UNICORN and regardless of the identity and access rights of the current user. Alarms and warnings can however only be acknowledged from the control mode connection.

If allowed by the system strategy, limits for certain monitor signals may also be set locally in a method, overriding the global setting as long as the method is in operation. This feature allows for instance the pH warning threshold to be set to one value during process operation and another during system cleaning.

Error messages from the monitors and pumps are reported if the respective **Error** settings are **Enabled**.

IMPORTANT!
Alarms are not active unless the mode is set to **Enabled**. This can be viewed for each instruction in the list.

The hysteresis setting (not available for ÄKTAdesign systems) for an alarm determines the extent to which the signal can oscillate around the warning limits without re-activating the warning (Figure 15-2).

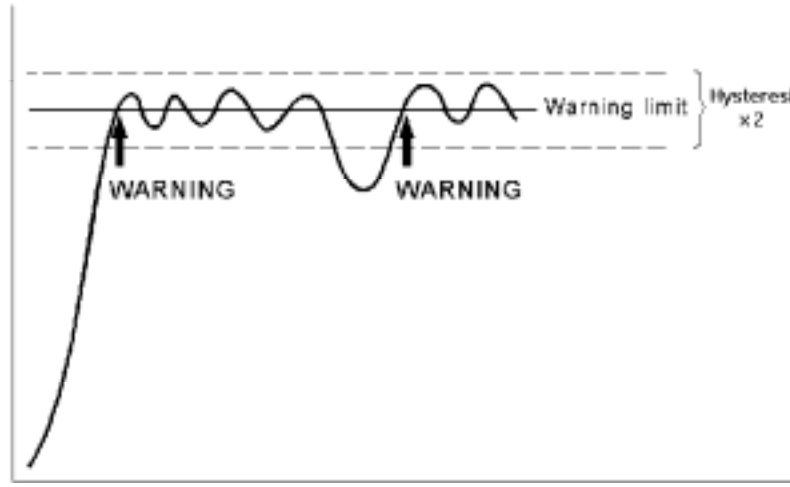


Figure 15-2. The hysteresis (not available for ÄKTAdesign systems) setting defines the limits within which the signal may oscillate up or down from the threshold without re-activating a warning. After the signal has activated a warning, the warning will not be repeated as long as the signal remains within a window defined by the hysteresis setting above and below the warning limit. This prevents repeated warnings from noisy or oscillating signals close to the warning boundary.

Hysteresis is only relevant for warnings, since an alarm puts the system in Pause at the first alarm.

15.2 Specials

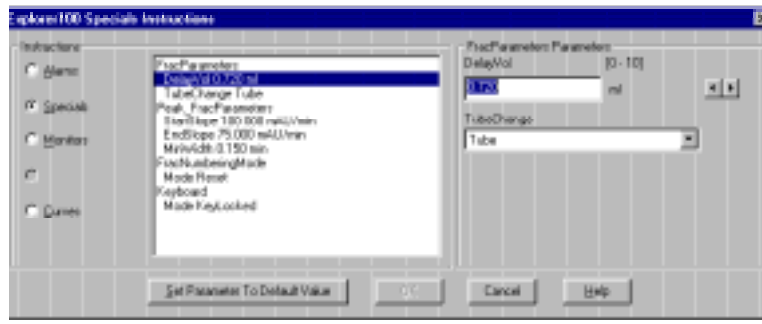


Figure 15-3. Special settings in System control.

Settings in the **Specials** group include:

Frac-Parameters

DelayVol determines the delay between peak detection on the UV monitor and the start of fraction collection. Fraction marks in the System control display and result file are adjusted with the delay volume. This value should be set to the volume of tubing between the UV monitor and the fraction collector outlet. **TubeChange** determines the flow handling during tube changes.

Peak_Frac-Parameters

Determines the parameters used for peak fractionation.

FracNum-bering Mode

Determines whether fraction numbers will be reset after each run (**Reset** or **Continue**).

Keyboard

Determines if the manual changes can be performed also from the instruments.

Methodbase

Determines which pump flow rate (GradientPump or SamplePump) will be used to calculate method volume.

PumpType

Only for BioPilot. Determines the types of pump used for pumps A, B and C.

PumpGain — Only for BioPilot. Sets the gain factor for the pump control signal. Normal value 1.0.

OutletValve — Available for some ÄKTAdesign systems. Determines if the outlet valve is connected or not.

15.3 Monitors

Monitor settings determine how baseline and peaks in the monitor signal are handled when Watch instructions are used (see Section 5.8.8), as well as a number of other settings for certain monitors such as whether the local keypad on the monitor can be used. For conductivity monitors, values for **SetCondScale0%** (0% eluent B) and **SetCondScale100%** (100% eluent B) must be set. There is no default for these values.



Figure 15-4. Monitors settings in System control.

Some of the settings in the **Monitors** group are:

Wavelength — Determines the default wavelengths.

Averaging time — Sets the default averaging time.

SetCondScale0%
SetCondScale100% — Sets the conductivity values in mS which correspond to 0% and 100% eluent B. These values must be provided for correct monitoring of eluent concentration (% of eluent B) by the Cond% curve.

CondTempComp Relates conductivity to concentration and/ or compares conductivity values. The compensation consists of a compensation factor together with a reference temperature (CondRefTemp). All conductivity values will then automatically be converted to the set reference temperatue. The factor is expressed in percentage increase of conductivity per degree Celsius increase in temperature. If the CompFactor is unknown a general approximate value of 2% can be set for many common salt buffers. 0%=Off.

CondRefTemp Selects the reference temperature to which the conductivity values will be converted. CondRefTemp is active when a factor of CondTempComp is selected.

pHCell This setting determines whether or not the pH Cell monitor is enabled.

pHTempComp Sets the temperature compensation on or off. For more accurate measurements during temperature changes, the pH measurement can be temperature compensated. When using pHTempComp it is important that the temperature of the pH electrode is the same as that of the conductivity flow cell since that is where the temperature is measured.

WatchPar The **WatchPar** settings for each monitor (e.g. **WatchPar_UV1**) includes **Delta_Peak** and **Delta_Base** values, which affect the way certain conditions for Watch instructions are interpreted. See Section 5.8.8 for details. For BioPilot, **WatchPar** settings are named **Peak**.

Keys

Only for BioPilot. The Keys settings (e.g. UV1Keys, pH1Keys) determine whether the keyboard can be used on the respective monitor. If the keyboard is disabled, the monitor can only be calibrated and controlled from UNICORN.

15.4 Curves

Curve settings determine which monitor signals will be stored as curves in the result file. Check that **Store** is set to **ON** for all signals that are to be monitored.

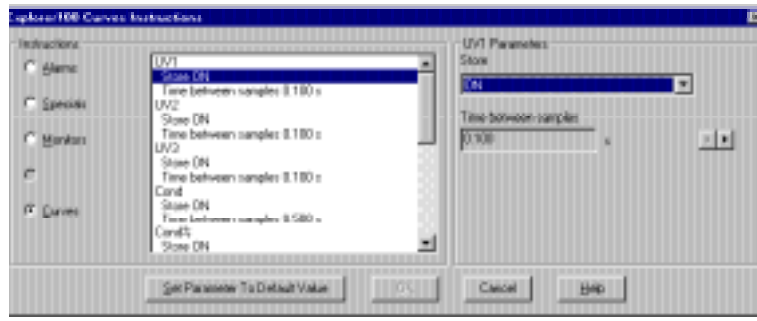


Figure 15-5. Curves settings in System control.

Settings in the **Curves** group include:

Store

Determines whether the curve data is recorded in UNICORN.

Time between samples

The **Time between samples** determines the frequency with which curve data is recorded in UNICORN (this does not affect the reading frequency of the monitor itself). Default values are the shortest possible time between samples.

CAUTION
If a curve is set to **Store:OFF**, data from the monitor concerned cannot be displayed in the curves window during a process run, and will not be recorded in any way.

Introductory material

Methods and runs

Evaluation

System management

Appendices

A Technical specifications

A.1 System requirements

A.1.1 Hardware requirements

- Compaq PC, Pentium/233 MHz or later (minimum Pentium/90 MHz)
- 64 Mb RAM (minimum 32 Mb) for one system
  92 Mb RAM (minimum 64 Mb) for two or more systems
- 1 Gb of available hard disk space, NTFS file system
- Colour monitor, 1024x768 pixels (minimum 800x600), small fonts, 64k colours
- 1 ISA slot per connected system
- CD-ROM drive
- 1.44 Mb (3.5") diskette drive
- Mouse
- Supported printers:
  HP DeskJet 660C
  HP DeskJet 690C
  HP DeskJet 870Cxi
  HP LaserJet 4M
  HP LaserJet 5MP
  HP LaserJet 6P

UNICORN 3.00 is tested on every computer model that can be ordered from Amersham Biosciences. Full support can not be Guaranteed if any other model is used

A.1.2 Software requirements

Microsoft Windows NT Workstation 4.0 (with Service Pack 3 or later).

A.1.3 Network requirements

The following are minimum network requirements for running UNICORN in a network installation:

- Supported Network cards:
  3COM Etherlink III
  Compaq Netelligent 10/100 TX Embedded UTP Controller
  Compaq Integrated NetFlex-3 Controller
  AMD PCNET PCI Ethernet Adapter (Integrated)
- Novell NetWare version 4.02 or later or Microsoft Windows NT Server 4.0.
- A valid network connection

A.2 Control capacity

A.2.1 Stand-alone installations

Simultaneous control of up to four chromatography systems. Each module is separately configured in a system strategy supplied by Amersham Biosciences.

A.2.2 Network installations

Up to four chromatography systems may be connected to each local station in the network. Each station (local or remote) may control up to four systems simultaneously. Up to 20 computers with UNICORN installed may be logged on to the network at the same time. Each local station supports up to 8 simultaneous connections throughout the network (if more than one chromatography system is connected to a local station, the total number of connections to the systems installed on that system may not exceed 8, regardless of which station the connections are made from).

A.3 Data sampling

Data from chromatography system monitors are stored temporarily in data buffers in the local system controller. Data are transferred from the buffers to disk storage by UNICORN whenever a chromatogram is closed (i.e. when the New_Chromatogram instruction is issued or the result file is closed). Data are also saved to disk at pre-set intervals during a run, minimising data loss in the event of power or communication failure.

The capacity of the data buffers is 16000 points for up to sixteen monitors (as listed in the **Curves** group of **System:Settings** in System control). If a buffer is filled during a run, the number of points is halved by deleting every second point, and the sampling frequency for subsequent points is halved. The initial sampling frequency for each monitor is set in the system strategy, and can be viewed and changed in the **Curves** group of **System:Settings**. For ÄKTAdesign systems, the sampling frequency for the UV curves is set to 10 samples/s (10 Hz). For BioPilot System strategies, the sampling frequency is normally set to 0.5 or 1 sample/s (0.5 or 1 Hz) depending on the type of monitor.

At an initial sampling frequency of 10 samples per second (10 Hz), the following resolutions apply for the curves:

Duration	Sampling frequency	No. of points	Resolution (s/point)
0-27 min	10 Hz	0-16000	0.1
27-53 min	5 Hz	8000-16000	0.2
53-107 min	2.5 Hz	8000-16000	0.4
107-203 min	1.25 Hz	8000-16000	0.8
...	...	...	...
...	...	...	...

To ensure maximum resolution for part of a run, issue a New_Chromatogram instruction at the beginning of the part. This empties the data buffers and resets the sampling frequency to that specified in the system settings.

B Strategy for ÄKTAexplorer 100

A dedicated strategy is available for each system in the ÄKTAdesign platform. The instructions available differ slightly between strategies, although the majority are common. To find a description of any instruction in your particular strategy, mark the instruction and click on <F1>.

As an example, this appendix lists the instructions for methods, manual control and system settings supported by the strategy for ÄKTAexplorer 100 (E100-110).

B.1 Method instructions

B.1.1 Pump

Instruction	*Description*	*Parameters*
Flow	Starts the flow for the gradient pump at the set flow rate	Flow rate (0-100 ml/min)
Gradient	Defines a linear gradient for gradient pump (pump A and B). Reaches the target value after the specified length. Length is expressed in time, volume or column volume depending on selected base.	Target (0-100 %B) Length (0-9999 base)
BufferPrep_pH	Selects pH for a defined BufferPrep recipe. When BufferPrep is not used, the instruction should be set to zero, otherwise an alarm will be given.	pH (0-14)
SampleFlow	Starts the flow of the sample pump at the setpoint flow rate. The sample pump can be calibrated in the calibration module.	Flow rate (0-5 ml/min)

PumpWash	PumpWash is used to change eluents in the inlet tubings, pump and mixer. THE PUMPS MUST BE PURGED PRIOR TO USING PUMPWASH. Flow is diverted to the waste position of the injection valve. Up to four inlets can be washed simultaneously; InletA1, InletA2, InletB1 or Inlet B2. One of the eight inlets of buffer valve A1 can be selected for InletA1. The system is set to Pause during PumpWash and the pressure limit is set to the value given in instruction Wash_alarm_pressure in **System:Settings**. After PumpWash the injection valve turns back to its previous position and the flow and pressure limits are set to their previous values.(Flow rate: 25 ml/min, Wash volume: 30 ml/inlet).	InletA1 OFF (A11-A18) InletA2 OFF ON InletB1 OFF ON InletB2 OFF ON
SystemWash	SystemWash is used to change eluents in the system, including inlet tubings, pumps, mixer and flow cells. THE PUMPS MUST BE PURGED PRIOR TO USING SYSTEMWASH. Flow is diverted to the waste at the outlet valve. The ColumnPosition is set to Position1Bypass during SystemWash. One inlet can be selected, A11-A18. When running BufferPrep methods the SystemWash instruction will deliver buffer with the selected composition (Recipe and BufferPrep_pH). The system is set to Pause during SystemWash and the pressure limit is set to the value given in instruction Wash_alarm _pressure in **System:Settings**. After SystemWash the OutletValve and ColumnValves turn back to their previous positions and the flow and pressure limit are set to their previous values.(Flow rate: 25 ml/min, Wash volume: 75 ml)	InletA1 (A11-A18)

SamplePump-Wash	SamplePumpWash is used to rinse the sample tubing and the sample pump. Flow from the sample pump is diverted via the injection valve in Load position to sample waste. The system is set to pause during sample pump wash. After sample pump wash the injection valve is turned back to its previous position (Flow 5 ml/min, WashVolume 5 ml)	Inlet (S1) OFF S1-S8
MethodBase	Sets the method volume calculation from either GradientPump or SamplePump	Base GradientPump SamplePump

B.1.2 Flowpath

Instruction	*Description*	*Parameters*
BufferValveA1	Selects the position for Buffer Valve A1. Position A11 corresponds to position 1 on the Buffer Valve A1, position A12 to position 2, etc.The flow rate is reduced to 0 ml/min when the valve is switched.	Position (A11-A18)
PumpAInlet	Sets the position of the pump A inlet. The instruction is not allowed in a method when running BufferPrep (gives an alarm in BufferPrep mode).	Position A1 A2
PumpBInlet	Sets the position of the pump B inlet. The instruction is not allowed in a method when running BufferPrep (gives an alarm in BufferPrep mode).	Position B1 B2
SampleValve	Selects the position for the Sample-Valve.	Position (S1-S8)
InjectionValve	Sets the Injection Valve positions. The Injection Valve is switched to position 1 with parameter "load", to position 2 with parameter "inject" and to position 3 with parameter "waste". The instruction gives an injection mark when the injection valve switches to the "inject" position.	Position Load Inject Waste

InjectionMark	The instruction gives an injection mark. The instruction is useful when the sample is introduced onto the column with the gradient pump.	-
ColumnPostion	Selects the position for the column. ColumnPosition switches the two column valves at the same time. Position1Bypass corresponds to a tubing connected to valve ports of same number and Position2 corresponds to a column connected to valve ports of same number, etc. The flow rate is reduced to 0 ml/min when the valves switch.	Position Position1Bypass, Position2-8)
FlowDirection	Sets the flow direction for the column. The instruction is useful when cleaning columns. The instruction switches the Flow Direction Valve to position 2 with parameter UpFlow and to position 1 with parameter DownFlow. The flow rate is reduced to 0 ml/min when the valve switches.	Direction Downflow Upflow
OutletValve	Selects the position for the Outlet valve. Feed switches the outlet valve one step forward from its actual position. The OutletValve gives fraction marks.	Position (WasteF1 FracF2 F3-F8 Feed)
OutletFractions	Enables collection of a number of fractions of equal volume in subsequent outlets from the Outlet Valve. Fractionation starts at the selected outlet. If more fractions are programmed than the number of outlets, an alarm will be given. After pressing continue the fractionation will resume in F3. After completing the last fraction the position selected before executing this command is restored.	How many? (0-7) Start at (F2-F8) Volume (0 - 100000 ml)
FractionCollector	Starts fraction collection with the fraction size specified in the FracSize parameter and set the outlet valve to F2. When a fraction mark is set on the screen, the tube rack moves on to the next tube after the set delay volume.	FracSize (0-50 ml)

Fractionation-Stop	Stops fraction collecting after the set delay volume has been collected. The flow is diverted to the waste of the outlet valve.	-
FeedTube	Moves the tube rack forward one tube after the set delay volume has been collected.	-
Peak_Frac-Parameters	Parameter settings for Peak_Fractionation. To obtain suitable values, perform a test run and measure the slope at the beginning and end of the smallest and flattest peak you want to collect, using **Operations: Differentiate** in Evaluation. Time (min) as base must be used. Min-width prevents collection of noise spikes. Enter a value of about half the width of the narrowest peak to collect.	StartSlope (0.01-10000 mAu/min) EndSlope (0.01-10000 mAU/min) MinWidth (0.01-1500 min)
Peak_Fractionation	Peak_Fractionation enables only peaks to be collected. Peak_ Fractionation uses UV1 to determine when there is a peak to collect. Collect the peak in one tube by setting PeakSize (=fraction size)>peak volume. Peak_ Fractionation will use the default peak fractionation parameters (Peak_ FracParameters), which can be found under menu **SystemControl:System:Settings: Monitors**, if Peak_ FracParameters are not set in the method. When a fraction mark is set the tube rack moves on to the next tube after the set delay volume. It is recommended that Peak fractionation is used together with normal fractionation, otherwise the volume remaining in the tubing to the fraction collector may contaminate the next peak.	PeakSize (0-50 ml)
Peak_FracStop	Stops the peak fraction collection after the set delay volume has been collected.	-

B.1.3 Alarms&Monitors

Instruction	*Description*	*Parameters*
Wavelength	Sets the wavelength and number of wavelengths to monitor. The range is 190-700 nm for UV1, UV2 and UV3. 0=OFF for UV2 and UV3. Wavelengths <190 nm are automatically set to 0=off.	UV1 (190-700 nm) UV2, 0=OFF (0-700 nm) UV3, 0=OFF (0-700 nm)
AveragingTime	Filters the noise in the UV-signal. Averaging time is the time interval used for calculating the moving average of the absorbance signal. A long averaging time will smooth out noise efficiently, but will also distort the peaks. Choose an averaging time of the greatest value that can be set which is less than (1/11)*60(s/min)*Typical peak width (ml) /flow rate (ml/min). The averaging time should not be less than 1.28s when more than one wavelength is monitored. A column-specific averaging time is automatically set when a column and template are selected.	AvTimeUV 0.01 0.02 0.04 0.08 0.16 0.32 0.64 1.28 2.56 5.12
AutoZeroUV	Sets the relative AU to zero.	-
SetCondScale 0%	Sets the 0% scale of the relative conductivity signal. The relative conductivity is displayed as a percentage of bufferB in run data (Cond%) and as a curve (Cond%).	CondValue (0-999.9 mS/cm)
SetCondScale 100%	Sets the 100% scale of the relative conductivity signal. The relative conductivity is displayed as a percentage of bufferB in run data (Cond%) and as a curve (Cond%).	CondValue (0-999.9 mS/cm)
Alarm_ Pressure Alarm_UV1 Alarm_Cond Alarm_pH	Sets the alarm limits for the signal. An alarm will set the system in Pause.	Mode Disabled Enabled HighAlarm LowAlarm

WatchPar_UV1 WatchPar_cond WatchPar_pH WatchPar_Pressure WatchPar_Conc	The Delta_Peak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction (see UNICORN User Manual chapter 5.8.8). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value above or below the time interval specified in the Stable_baseline condition in the watch instruction.	Delta_Peak Delta_Base

B.1.4 Watch

Instruction	*Description*	*Parameters*
Watch_Pressure Watch_Conc Watch_UV Watch_pH Watch_Cond	Sets a watch on the signal (UV1 for UV), to perform a set action if a given test condition is fulfilled. The watch is cancelled automatically when the condition has been fulfilled once.	Test * (see below) Value Action * (see below) Value
Watch_Off	Cancels a watch on a specified monitor.	Signal Pressure Conc UV pH Cond
*Test Greater_than Less_than Slope_greater_than Less_than_or_ valley Peak_max Stable_baseline	*Action Block names Ready Pause Hold End_Method End_Block Continue	

B.1.5 Other

Instruction	*Description*	*Parameters*
Base	Defines the base for a block for calculating breakpoints. Each block must have a defined base.	Column Any/column name) Base Time Volume (ml) CV SameAsMain
Block	Calls a block unconditionally.	Block name
Continue	Resumes execution of a paused or held method. This instruction has the same effect as clicking on the Cont. button in System control.	-
End_Method	Terminates method execution, equivalent to clicking on the End button in System control.	-
Evaluate	Calls an evaluation procedure. The procedure must be stored together with the current method.	Procedure name
Hold	Places the system in Hold state. This instruction has the same effect as clicking on the Hold button in System control.	-
Loop	Runs the instructions between Loop and Loop_end for the specified number of times.	No of loops (1 - 9999)
Loop_end	Specifies the end of the instructions contained within a loop.	-
Message	Generates a user-defined message which is recorded in the log book and may be displayed on the screen.	"Message" Mode: Screen / Noscreen
New_ chromatogram	A new chromatogram is created within the result file and all data collected after this instruction is stored within the newly created chromatogram until another New_ chromatogram instruction is issued.	Name

Pause	Places the system in the Pause state for the specified length of time.	Time (-1 (infinite) - 9999.9 in minutes)
Ready	Indicates that the next step in a MethodQueue may start.	-
End_Block	Terminates a block and returns control to the point from which the block was called.	-
;	Inserts a comment in the method below the marked instruction.	Comment

B.2 Manual control

B.2.1 Pump

This group contains the same instructions as the Pump group in method instructions (Section B.1.1).

B.2.2 Flowpath

This group contains the same instructions as the Flowpath group in method instructions (Section B.1.2) with the addition of the following instruction:

Instruction	*Description*	*Parameters*
ResetFracNumber	Sets the tube number to 1.	-

B.2.3 Alarms&Monitors

This group contains the same instructions as the Alarms&Mon group in method instructions (Section B.1.3).

B.2.4 Other

Instruction	*Description*	*Parameters*
Block	Calls a block. Only available when a method is running.	Block Block name
Next_ breakpoint	Jump to the next breakpoint in the current method (only relevant when a method is running).	-
BufferPreprecipe	Select a recipe for running BufferPrep manually. The recipe must be selected as the first instruction.	Recipe Name
Record_on	Begins recording a run that has been started manually. A result file will be generated.	-

B.3 System settings instructions

B.3.1 Alarms

Instruction	*Description*	*Parameters*
Alarm_ pressure Alarm_UV1 Alarm_Cond Alarm_pH	Sets the alarm limits for the signal. An alarm will set the system in Pause.	Mode Disabled Enabled HighAlarm LowAlarm
Wash_Alarm _pressure	Sets the alarm limits for pressure when instruction PumpWash or SystemWash are used. This pressure limit only applies when these instructions are executed.	Mode Disabled Enabled High Alarm 0-10 MPa Low Alarm 0-5 MPa

B.3.2 Specials

Instruction	*Description*	*Parameters*
FracParameters	Parameter settings for fraction collector. Delay volume is defined as the volume from UV to outlet of the fraction collector. The sample flow during tube change can be handled in different ways. Tube: No synchronization of collection. Dropsync: Tube change synchronized to drop release.	DelayVol (0-10 ml) Tubechange DropSync
Peak_FracParameters	Parameter settings for Peak_Fractionation. To obtain suitable values, perform a test run and measure the slope at the beginning and end of the smallest and flattest peak you want to collect, using Operations: Differentiate in Evaluation. Time (min) as base must be used. Minwidth prevents collection of noise spikes. Enter a value of about half the width of the narrowest peak to collect.	StartSlope (0.01-10000 mAu/min) EndSlope (0.01-10000 mAU/min) MinWidth (0.01-1500 min)
FracNumbering Mode	Determines whether fraction numbering is reset at the end of a method. Reset sets Tube No to 1 after method end. Continue continues counting from the last tube number in the previous method.	Mode Reset Continue
Keyboard	Sets the mode of the user interface of the instruments.	Mode Open KeyLocked KeyAndDialLocked

B.3.3 Monitors

Instruction	*Description*	*Parameters*
Wavelength	Sets the wavelength and number of wavelengths to monitor. The range is 190-700 nm for UV1, UV2 and UV3. 0=OFF for UV2 and UV3. Wavelengths <190 nm are automatically set to 0=off.	UV1 (190-700 nm) UV2, 0=OFF (0-700 nm) UV3, 0=OFF (0-700 nm)
AveragingTime	Filters the noise in the UV-signal. Averaging time is the time interval used for calculating the moving average of the absorbance signal. A long averaging time will smooth out noise efficiently, but will also distort the peaks. Choose an averaging time of the greatest value that can be set which is less than (1/11)*60(s/min)*Typical peak width (ml) /flow rate (ml/min). The averaging time should not be less than 1.28s when more than one wavelength is monitored. A column-specific averaging time is set automatically when a column and a template are selected.	AvTimeUV 0.01 0.02 0.04 0.08 0.16 0.32 0.64 1.28 2.56 5.12
SetCondScale 0%	Sets the 0% scale of the relative conductivity signal. The relative conductivity is displayed as a percentage of bufferB in run data (Cond%) and as a curve (Cond%).	CondValue (0-999.9 mS/cm)
SetCondScale 100%	Sets the 100% scale of the relative conductivity signal. The relative conductivity is displayed as a percentage of bufferB in run data (Cond%) and as a curve (Cond%).	CondValue (0-999.9 mS/cm)

CondTempComp	Relates conductivity to concentration and/or compares conductivity values. The compensation consists of a compensation factor together with a reference temperature (CondRefTemp). All conductivity values will then automatically be converted to the set reference temperature. The factor is expressed in percentage increase of conductivity per °C increase in temperature. If the CompFactor is unknown, a general approximate value of 2% can be set for many common salt buffers. 0% = off.	CompFactor (0-9.9%)
CondRefTemp	Selects the reference temperature to which the measured conductivity values will be converted. CondRefTemp is active when a factor of CondTempComp is selected.	RefTemp (0-99.9°C)
pHCell	Reports values of pH to Run data and flow scheme in System control. Install the pH according to pH/C-900 User Manual. Set pHCell to Connected:Yes to activate the reporting.	Connected Yes No
pHTempComp	Sets the temperature compensation on or off. For more accurate measurements during temperature changes, the pH measurement can be temperature compensated. When using pHTempComp it is important that the temperature of the pH electrode is the same as that of the conductivity flow cell since that is where the temperature is measured.	pHTempComp ON OFF

WatchPar_UV1 WatchPar_Cond WatchPar_pH WatchPar_Conc WatchPar_Pressure	The Delta_Peak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction (see UNICORN User Manual chapter 5.8.8). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value above or below the time interval specified in the Stable_baseline condition in the watch instruction.	Delta_Peak Delta_Base

B.3.4 Curve

Instruction	*Description*	*Parameters*
UV1 UV2 UV3 Cond Cond% Conc pH Pressure Flow Temp	Set the specific signal curve on/off for storing in the Result file. The time between samples determines the frequency with which curve data is recorded	Store ON / OFF Time between samples

C Systems using the UNICORN controller box

This appendix lists examples of method instructions for systems using the UNICORN controller box. System-specific instructions should be obtained from the system manual.

C.1 Method instructions

C.1.1 Pump

Instruction	*Description*	*Parameters*
FlowAB	Starts flow of pump A and B at the setpoint flowrate	Setpoint (0 – 100 ml/min)
Gradient	Defines a linear gradient between pump A and B	Target (0 – 100 %B) After (0 – 99999 Base)
FlowC	Starts flow of pump C at the setpoint flowrate	Setpoint (0 – 100 ml/min)
Methodbase	Sets the method volume calculation from either FlowAB or FlowC.	From: AB / C
Doubleflow	Sets the Doubleflow function on/off. When set to on, FlowAB setting is sent to both pumps creating a double flow. Note: The gradient formation is disabled when Doubleflow is on.	Mode: On / Off

Available for all strategies except Valve panel Strategy:

WashA	Switches valve 1 to position 3,	–
WashB	WASH AB, when A or B is selected.	–

WashC	Valve 1 goes to position 2, WASH C,if C is selected. Starts the "wash" programme within the pump. Valve returns to previous position after "wash" is completed.	–
SyncPumpA	Moves the pump head of the	–
SyncPumpB	respective pump to the end position.	–
SyncPumpC	Valve 1 is set as for WashX.	–

Only available in Valve Panel Strategy:

WashA	Starts wash program in pump.	–
WashB	Injection valve must be set to position	–
WashC	WASH (3).	–
SyncPumpA	Moves the pump head of the	–
SyncPumpB	respective pump to the end position.	–
SyncPumpC	Injection valve must be set to position WASH (3)	–

C.1.2 Flowpath

Instruction	*Description*	*Parameters*
InletA[1])	Switches valve 4 to the selected inlet.	A1, A2, A3, A4, A5, A6, A7, A8
InletB[1])	Switches the valve to the selected inlet	B1, B2 or B1-B8
InletC[1])	Switches the valve to the selected inlet	C1, C2 or C1-C8

SampleLoop	Switches valve1 to position 1 with parameter "load" and to position 2 with parameter "inject". Method Development 2 and Pilot Work strategies also support parameter "PumpCDirect" which allows direct application of sample to the column using pump C. This mode switches valve 1 to position 2 and valve 7 (Pilot work systems) or valve 8 (Method Development 2 systems) to position 3.	Mode: Load /Inject / PumpCDirect
Superloop[2])	In "load" valve 1 is set in position 1 and pump C (Flow C) is used to fill Superloop with the set volume. When the set volume is reached Flow C is set to 0. In "inject" valve 1 is set to position 2 and the volume is injected using Flow AB. After the volume is reached valve 1 is changed from position 2 to position 1.	Volume: 0 - 150 ml) Mode: Load / Inject
Column[1])	Switches the two column valves 2 and 3. Bypass 1 corresponds to a column bypass in valve position 1. Column 2 etc., corresponds to the column connected to the valve ports of same number. "Direction" (not available in laboratory strategy) switches valve 5 to position 1, (downflow) or position 2 (upflow).	Macro: ByPass_1, Column 2, 3, 4, 5, 6, 7, 8 Direction: Downflow / Upflow
Outlet[3])	Switches valve 6 to the selected outlet.	F1, F2, F3, F4, F5, F6, F7, F8
Collect	Activates the fraction collector and starts fraction collection with the fraction size specified in the Collect instruction. UNICORN sends a signal to the fraction collector for each new fraction. The following parameters must be set on the fraction collector: Collection mode: Time Delay time or volume: 0 Fraction size: highest possible (e.g. 9999 min for FRAC-100 and FRAC-200, 99 h for SuperFrac).	Fraction size (0- 10000ml)

CollectEnd	Ends fraction collection immediately and advances to the next tube. The delay volume is not taken into account. A method should be allowed to run for at least 10 s after Collect-End to ensure correct fraction numbering.	
Fractions[3])	Creates a number of fractions of equal volume in subsequent outlets. If more fractions are programmed than number of outlets the fractionation will continue in the first fractions. After completing the last fraction the position selected before executing this command is restored.	How many? (0-7) Volume: (0 - 100, 000 ml) Start at: F2, F3, F4, F5, F6, F7, F8
StepValve1* StepValve2 StepValve3 StepValve4 StepValve5 StepValve6 StepValve7 StepValve8	Moves the valve one position forward from actual position. The instruction works with MV7 and MV8 valves. (* only in Valve Panel strategy)	
Frac	Used to start and stop fraction collector Frac-100, Frac-200 or SuperFrac connected to the system. The fraction size and delay time must be programmed on the fraction collector. Do not use any of the Frac parameters in combination with the instruction Collect.	Action: Start, Stop, Pause_Frac, Continue_Frac, Feed_Tube
Valve1 Valve2 Valve3 Valve4 Valve5 Valve6 Valve7 Valve8	Sets the valve in the selected position. Valve 1 – 8 can be MV7 or MV8. MV7 can be set to position 1 – 3. MV8 can be set to position 1 – 8.	Position: (1 -8) for all

1. Not available in valve panel strategy
2. Only available in multipurpose strategy
3. Not available in Laboratory and Valve Panel strategies

Only available in Valve Panel Strategy:

ValveA Valve B	Sets valve PSV-50 in the selected position	Position: A1, A2 Position: B1, B2 Position 1 = IN – NO Position 2 = IN – NC

C.1.3 Alarms&Monitors

Instruction	*Description*	*Parameters*
Mark	Sets a mark anywhere in the chromatogram to show e.g. events such as injection or a valve switch. Several marks can be set.	-
AutozeroUV	Autozeroes the UV monitor. Relative AU is set to 0 when executed.	-
pABAlarm	Sets the alarm and warning limits for the pressure on pump A signal.High alarm (0 - 40 bar) An alarm will set the system in Pause. A warning will issue a warning message with the system in Run.	Low alarm (0 - 40 bar) High warning (0 - 40 bar) Low warning (0 - 40 bar) Hysteresis (0 - 5 bar) Mode: Disabled/Enabled
AS1Alarm	Enables/disables the Air sensor 1 alarm.	Disabled / Enabled
AS2Alarm	Enables/disables the Air sensor 2 alarm (optional).	Disabled / Enabled
PumpError	Enables/disables the alarms from the pumps.	PumpA: Disabl. / Enabl. PumpB: Disabl. / Enabl. PumpC: Disabl. / Enabl.

UV1Alarm pH1Alarm Cond1Alarm CondPAlarm	Sets the alarm and warning limits for the signal from the respective monitor.	As for pABAlarm (AU) (pH) (mS/cm) (%)
PeakUV1 PeakpH1 PeakCond PeakCond%	Sets the minimum increase to be regarded as a peak (MinPeak) when using Watch instructions, and the limits (±D_Baseline) used by the instruction Watch Stable_Baseline for the respective monitor signal.	MinPeak (unit) D_Baseline (unit)

C.1.4 Watch

Instruction	*Description*	*Parameters*
Watch_Cond1 Watch_CondP Watch_pH1 Watch_UV1 Watch_pAB	Sets a watch on a specified monitor signal, to call a block if a given test condition is fulfilled. The watch is cancelled automatically when the condition has been fulfilled once.	Test * (see below) Action† (see below)
Watch_AS2 Watch_AS1	Sets a watch on the specified air sensor signal	Equal 0 / 1 0 = No air 1 = Air Action† (see below)
Watch_off	Cancels a watch on a specified monitor.	pH1, UV1, Cond1, CondP AS2, AS1, pAB
	*Test: Greater_than Less_than Slope_greater_than Slope_less_than Less_than_or_valley Peak_max Stable_baseline†	 Value (unit) Value (unit) Slope (unit/min) Slope (unit/min) Value (unit) Factor (0-1, 1=detect peak max) Time (min)

	Action Continue End_block End_method Hold Pause Ready Call <block name>	

C.1.5 Other

Instruction	*Description*	*Parameters*
Base	Defines the base for a block for calculating breakpoints. Each block must have a defined base.	Column (None/column name) Volume (ml) Base: Time Volume CV SameAsMain
Call	Calls a block unconditionally. In Normal mode the system settingsremain unchanged when the block is terminated by End_Block. In Restore mode the current system settings are saved when the block is called and restored when the block is terminated by End_Block.	Block name Mode: Normal / Restore
Continue	Resumes execution of a paused or held method. This instruction has the same effect as clicking on the Cont. button in System control.	-
End_Method	Terminates method execution, equivalent to clicking on the End button in System control.	-
Evaluate	Calls an evaluation procedure. The procedure must be stored together with the current method.	Procedure name

Hold	Places the system in Hold state. This instruction has the same effect as clicking on the Hold. button in System control.	-
Loop Loop_end	Runs the instructions between Loop and Loop_end for the specified number of times.	No of loops (1 - 9999)
Message	Generates a user-defined message which is recorded in the process log and may be displayed on the screen.	"Message" Mode: Screen / Noscreen
New_chromatogram	Open a new chromatogram icon in the result file. All data collected after the instruction will be stored under the new icon until another New_chromatogram instruction is issued.	Chromatogram name
Pause	Places the system in the Pause state for the specified length of time.	Time (-1 (infinite) - 9999.9 in minutes)
Ready	Indicates that the next step in a MethodQueue may start.	-
End_Block	Terminates a block and returns control to the point from which the block was called.	
;	Inserts a comment in the method below the marked instruction.	Comment text

C.2 Manual control instructions

C.2.1 Pump

This group contains the same instructions as the Pump group in method instructions (Section C.1.1).

C.2.2 Flowpath

This group contains the same instructions as the Flowpath group in method instructions (Section C.1.2) with the addition of the following instruction:

Instruction	*Description*	*Parameters*
SetTubeNo1	Sets the fraction number to 1 even if ResetCount is set to OFF in system settings.	

C.2.3 Alarms&Mon

This group contains the same instructions as the Alarms&Mon group in method instructions (Section C.1.3) with the addition of the following instructions:

Instruction	*Description*	*Parameters*
UV1Runhour pH1Runhour Cond1Runhr	Resets the time counter for operation o f the respective monitor.	Reset: No / Yes
S_Version	Displays the strategy version as a warning message	-

C.2.4 Other

Instruction	*Description*	*Parameters*
Call	Call of block.	Block name
Next break-point	Jump to the next breakpoint in the current method (only relevant when a method or block is running).	
Record on	Store the run in a result file.	-

C.3 System settings instructions

C.3.1 Alarms

Instruction	*Description*	*Parameters*
pABAlarm	Sets the alarm and warning limits for the pressure on pump A signal. An alarm will set the system in Pause. A warning will issue a warning message with the system in Run.	High alarm (0 - 40 bar) Low alarm (0 - 40 bar) High warning (0 - 40 bar) Low warning (0 - 40 bar) Hysteresis (0 - 5 bar) Mode: Disabled/Enabled
AS1Alarm	Enables/disables the Air sensor 1 alarm.	Disabled / Enabled
AS2Alarm	Enables/disables the Air sensor 2 alarm (optional).	Disabled / Enabled
PumpError	Enables/disables the alarms from the pumps.	PumpA: Disabl. / Enabl. PumpB: Disabl. / Enabl. PumpC: Disabl. / Enabl.
UV1Alarm pH1Alarm Cond1Alarm CondPAlarm	Sets the alarm and warning limits for the signal from the respective monitor.	As for pABAlarm (AU) (pH) (mS/cm) (%)
Cond1Error pH1Error UV1Error	Enables/disables all alarms from the respective monitor.	Disabled / Enabled
V5_V8Alarm	Enables/disables alarm for valve 5 to 8 respectively. An alarm is issued when no valve is present or when valve fails to reach position.	Disabled / Enabled

V1_V4Alarm	Enables/disables alarm for valve 1 to 4 respectively. Disabled / Enabled An alarm is issued when no valve is present or when valve fails to reach position.	Disabled / Enabled Disabled / Enabled Disabled / Enabled

C.3.2 Specials

Instruction	*Description*	*Parameters*
ResetCount	Determines whether fraction numbering is reset at the end of a method. ON resets numbering so that fraction numbers start at 1 for each new run.	Mode ON / OFF
FracDelay	Sets the delay volume between the UV cell and the fraction collector outlet. Fraction marks are adjusted by the delay volume when the Collect instruction is used.	Volume (ml)
Methodbase	Sets the method volume calculation from either FlowAB or FlowC.	AB / C
PumpGain	Sets the gain on the pump control signals. Used for fine-tuning of the pump speed. Default value is 1.00.	GainA (0.8 - 1.05) GainB (0.8 - 1.05) GainC (0.8 - 1.05)
PumpType	Sets the pump type connected to A, B, C.	PumpA * PumpB * PumpC ** (P1, P50, P500 , P6000)
PCFail	Defines the action to be taken if communication is lost between the PC and the system control unit. Continue means that the method will run to completion (note that no results will be saved after the communication failure). Stop means that the run will stop immediately.	Continue / Stop

Only available in Valve Panel strategy

Instruction	*Description*	*Parameters*
InjectVlv	Defines the sample injection valve. The SampleLoop instruction will control this valve.	

C.3.3 Monitors

Instruction	*Description*	*Parameters*
PeakCond1 PeakCondP PeakpH1 PeakUV1 Peak_p	Sets the minimum peak (MinPeak) not to be regarded as a disturbance when using the instruction Watch, and the limits (± D_Baseline) used by the instruction Watch Stable_baseline.	MinPeak (unit) D_Baseline (unit)
Cond1_100P	Sets the conductivity value corresponding to 100% eluent B.	Value 100% (mS/cm)
Cond1_0P	Sets the conductivity value corresponding to 0% eluent B.	Value 0% (mS/cm)
Cond1Keys pH1Keys UV1Keys	Enables/disables the keyboard on the respective monitor.	Disabled / Enabled

C.3.4 Curves

Instruction	*Description*	*Parameters*
UV1 pH1 Cond1* CondP* PressureAB FlowAB Gradient	Set the specific signal curve on/off for storing in the result file.	Store: ON / OFF Store: ON / OFF Store: ON / OFF Store: ON / OFF Store: ON / OFF Store: ON / OFF Store: ON / OFF

*Cond1 is the conductivity in mS/cm. CondP is the eluent concentration in % eluent B as determined from the conductivity.

D Evaluation functions and instructions

This appendix describes the functions implemented in the evaluation module. There are four sections in the appendix:

D.1 describes how the smoothing functions are calculated

D.2 gives an basic introduction into baseline calculation theory which is an essential part of peak integration

D.3 describes the peak table column components

D.4 the Procedure Editor instruction types are described which are used to build up an evaluation procedure

D.1 Smoothing algorithms

D.1.1 Moving Average

For each data point in the source curve, the processed curve is calculated as the average of the data points within a window centred on the source data point. The width of the window is determined by the parameter value, expressed as number of data points.

When the source point is less than half the window size from the beginning or the end of the curve, the average is calculated symmetrically round the source point over as many data points as possible.

Increasing the window width increases the smoothing effect.

The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given it is incremented by one.

D.1.2 Autoregressive

The first data point in the source curve is copied to the processed curve. For each subsequent data point, the previous processed point is multiplied with the parameter value and added to the current source data point. The result is then divided by the parameter value plus 1 according to the following formulae:

$$t_1 = S_1$$
$$t_n = \frac{(p * t_{n-1} + S_n)}{(p + 1)}$$

where

t_n = current processed point

t_{n-1}= previous processed point

S_n = current source point

p = smoothing parameter value

Increasing the parameter value increases the smoothing effect. The filter algorithm accepts integer parameter values between 1 and 25.

D.1.3 Median

For each data point in the source curve, the processed curve is calculated as the median of the data points within a window centred on the source data point. The width of the window is determined by the parameter value, expressed as number of data points.

When the source point is less than half the window size from the beginning or the end of the curve, the median is calculated symmetrically round the source point over as many data points as possible.

Increasing the window width increases the smoothing effect. To completely remove a noise spike, the window width should in principle be slightly more than twice the width of the spike.

The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given it is incremented by one.

D.2 Baseline calculation theory

The baseline calculation can schematically be described in three steps:

1. Find baseline segments

2. Select baseline points

3. Draw the baseline.

D.2.1 Finding baseline segments

In the first step, four baseline parameters are used to find the baseline segments. The parameters can be seen in the **Integrate:Calculate baseline** function or by pressing the **Baseline settings** button in the **Integrate:Peak integrate** function. The default values for the parameters are determined from the source curve.

The baseline segments are found by searching for all parts of the source curve which

1. are longer than the **Shortest baseline segment**.

   This parameter determines the minimum length for a part of the source curve to be considered a possible baseline segment.

2. have no point outside the **Noise window**.

   The noise window is defined as a rectangular corridor parallel to the slope of the curve and centred on the first and last points within the currently inspected segment.

3. slope less than the **Slope limit**.

   This limits the maximum slope of the baseline to differentiate baseline segments from peaks.

4. are lower than the **Max baseline level**.

   Determines the highest acceptable signal level for the baseline. This parameter is by default set to have no influence on the baseline calculation and is seldom necessary to adjust.

The parameters can be illustrated as a rectangular box in which the source curve has to fit to be identified as a baseline segment, see Figure D-1. The length of the box corresponds to the **Shortest baseline segment** and the height of the box corresponds to the maximum level of noise on the baseline segments and is referred to as the **Noise window**.

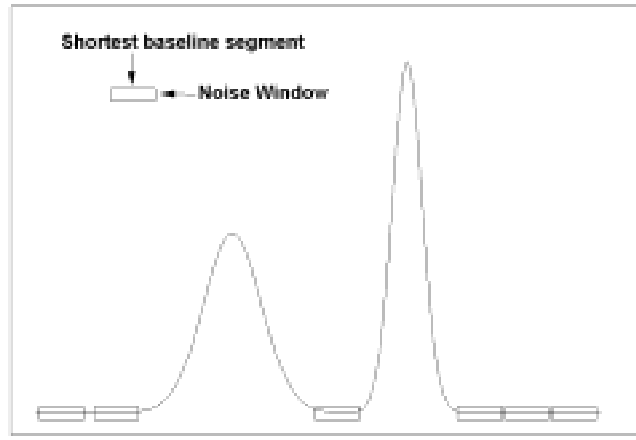


Figure D-1. Baseline box with Shortest baseline segment and Noise window.

The rectangular box is allowed to be tilted with a maximum slope corresponding to the **Slope limit**, see Figure D-2. The box is not allowed to move up above the **Max baseline level**.

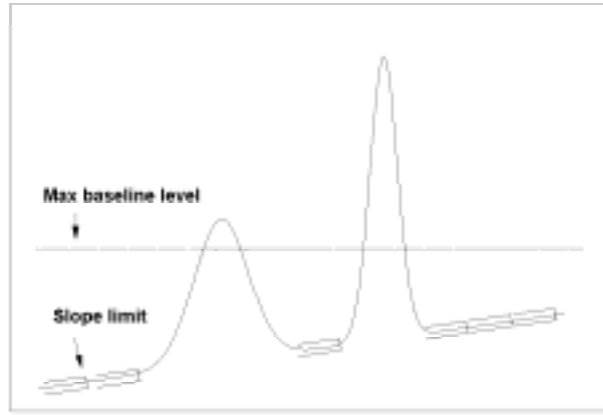


Figure D-2. Slope limit and Max baseline level.

When looking for baseline segments, the box is virtually moved along the source curve in steps of 1/3 of the **Shortest baseline segment**. A baseline segment is found whenever the currently examined part of the source curve fits completely within the box.

The found baseline segments are joined by connecting adjacent segments, provided that the slope of the joining lines does not exceed the **Slope limit**.

D.2.2 Selecting baseline points

In the second step, the baseline segments are replaced by a large number of baseline points. A baseline point is placed at the start and end of each segment. The line between these will also be filled with points. The baseline points are shown as pale blue crosses in the **Integrate:Edit baseline** function (see Section 10.1.5).

D.2.3 Drawing the baseline

The baseline points are used to create the baseline curve using a spline interpolation. The spline function ensures that the baseline curve is guided by the baseline points, but the curve does not necessarily pass through them. The baseline will thus be a smoothly curved function passing close to or through the points. To reduce the effect of noise on the peak integration, the created baseline is adjusted by forcing it equal to the source curve in every position where the difference between the baseline and the source curve is small enough. If the **Accept negative peaks** option (see Section 10.1.4) is off, the baseline will be forced down to the level of the source curve whenever the created baseline goes above the source curve.

D.2.4 Estimating the baseline parameters from the source curve

The baseline parameters can sometimes be difficult to set. Rough estimates can be found by analysing the source curve.

Measuring the Shortest baseline segment using curve co-ordinates

If you are uncertain of the length of the **Shortest baseline segment**, you can try to measure it directly on your chromatogram. Locate the shortest segment of the curve that you consider as a part of the baseline and measure the length of the segment using the **XY** box on the chromatogram (see Section 10.1.7). Insert this value as the **Shortest baseline segment** value.

Measuring the noise level using curve co-ordinates

As for measuring the Shortest baseline segment, curve co-ordinates can be used in exactly the same manner to measure noise levels on the source curve. First use the Zoom function to select a part of the curve representative of the baseline noise. Measure the Y-axis co-ordinates, using the appropriately selected Y-axis scale, to calculate the noise range as the difference between the max. and min values. Add an extra 20% and insert this value as the **Noise window** value.

D.2.5 Measuring the Slope limit using Differentiate and curve co-ordinates

To measure the slope at any point on the curve:

1. Select **Operations:Differentiate**. A dialogue will appear.

2. Select the desired source curve, check that a **First order calculation** is selected and click on **OK**. The differentiated curve will appear in the active chromatogram.

3. Measure the Y-axis values on the differentiated curve using the **XY** curve co-ordinates function. Remember to select the appropriate Y-axis scale. Any Y-axis value is interpreted as the UV curve slope at the selected retention point.

   If the differentiated curve is very noisy, it can be filtered using a light Moving average filter in the **Operations:Smooth** function (see Section 9.3.2)

4. Determine the highest slope value of the baseline (non-peak) part of the curve. Add an extra 10% and insert this value as the **Slope limit**.

D.3 Peak table column components

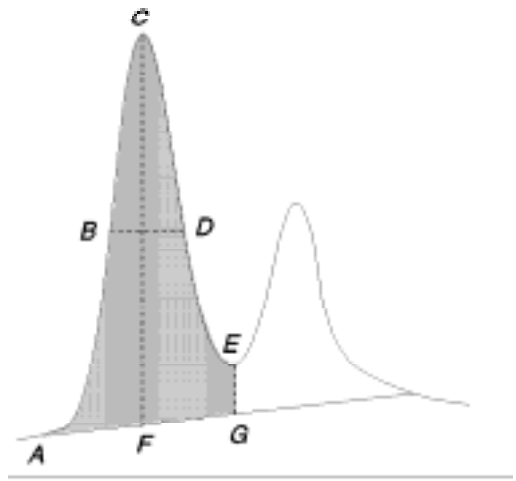


Figure D-3. Diagram illustrating peak parameters. See the parameter list below for explanations.

Peak name	Name of peak.
Retention	(time or volume base) Retention at the peak maximum (C in Figure D-4).
Width	(time or volume base) Difference in retention between the peak end and peak start (G-A in Figure D-4).
Area	(time or volume base) Calculated as the area between the curve and baseline, between the peak start and peak end (shaded in Figure D-4).
Height	Maximum amplitude above the baseline (C-F in Figure D-4).
Peak endpoint retention	(time or volume base) Retention value at peak start and peak end (A, G in Figure D-4).
Width at half height	(time or volume base) Calculated by taking the maximum height of the peak above the baseline, then determining the peak width at half this value above baseline (D-B in Figure D-4, where BD bisects CF).
Percent of total area	(time or volume base) Peak area as a percent of the total area under the curve above the baseline. Note that this value may differ in time and volume base if the flow rate is not constant throughout the method.
Percent of total peak area	(time or volume base) Peak area as a percent of the sum of all integrated peaks. Note that this value may differ in time and volume base if the flow rate is not constant throughout the method.
Type of peak limits	Identifies the criteria for peak start and peak end as either the baseline intersection or drop-line to the baseline.
Peak endpoint heights	Amplitude above the baseline at left (A in Figure D-4) and right peak limits (E-G in Figure D-4).
Fraction tube id	Fraction number at peak start, peak maximum and peak end.
Baseline height	Baseline amplitude at peak start, peak maximum and peak end (A, F and G in Figure D-4).
Sigma	Standard deviation for a Gaussian-shaped peak. For definition see below*

Resolution	Peak resolution. For definition, see Section 10.1.10 and below**.
Capacity factor	For a definition, see below ***. The Capacity factor will only be calculated when the chromatogram is in volume base. The total liquid volume, Vt, must be entered in the **Integrate** dialogue for this parameter to be calculated.
Kav	Gel phase distribution constant in gel filtration. For definition, see below ****. Kav will only be calculated when a gel filtration column was used and when the chromatogram is in volume base. The void volume, V0, must be entered in the **Integrate** dialogue for this parameter to be calculated.
Plate height (HETP)	Height equivalent to theoretical plate and plates/ metre. The column height must be entered in the Integrate dialogue for this parameter to be calculated. For definition, see Section 10.1.8 and below‡.
Asymmetry	Peak asymmetry (indicator of column packing). For definition, see Section 10.1.9 and below†.
Concentration	TBA
Amount	TBA
Molecular size	TBA

***Sigma**

$$\text{Sigma} = \frac{\sum (Y_i \, (i - i_{max})^2)}{A_{peak}}$$

where:

Y_i is the amplitude at retention sample number i on the peak

i_{max} is the sample number of the peak maximum

A_{peak} is the peak area.

The peak width for a Gaussian peak is (4 x Sigma).

****Resolution**

$$R = \frac{(V_{R2} - V_{R1}) \times 1.177}{w_{h1} + w_{h2}}$$

where:

V_{R1} = retention volume for peak 1

V_{R2} = retention volume for peak 2

w_{h1} = peak width at half height for peak 1 (for Gaussian peaks)

w_{h2} = peak width at half height for peak 2 (for Gaussian peaks)

***** Capacity factor**

$$k' = \frac{V_R - V_t}{V_t}$$

where:

V_R = retention volume

V_t = total liquid volume

****** K_{av}**

$$k_{av} = \frac{V_R - V_0}{V_C - V_0}$$

where:

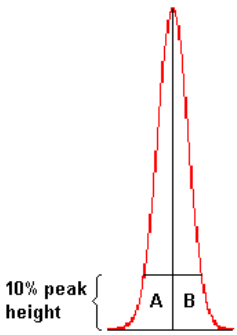
V_R =retention volume

V_0 = void volume

V_C = column volume

† Asymmetry

Asymmetry = width B / width A, where A and B are the partial peak widths measured at 10% of the peak height, with A representing the leading part of the peak and B the tailing part of the peak.



‡ HETP

HETP = L/N

$N = 5.54 * (V_R/w_h)^2$

where

N = no. of theoretical plates

L = bed height in cm

V_R = peak retention volume or time

w_h = peak width at half height expressed in the same units as V_R

D.4 Evaluation procedure

D.4.1 Curve operations

Instruction	*Description*	*Parameters*
ADD	Adds two curves to gain a third curve which is the sum of the two curves. The two source curves must have the same y-axis unit and not be fraction or injection curves or else a run time error will occur.	First source curve Second source curve Target curve position
AMP_MUL	Multiplies the amplitude of the source curve by the multiplication factor and stores the result in the target curve position.	Source curve Target curve position Multiplication factor
AMP_SHIFT	Shifts the amplitude of the source curve by the shift factor and stores the result in the target curve position.	Source curve Target curve position Shift factor
CLEAR	Clears specified curve from the working memory of the computer.	Source curve
COPY	Copies the source curve to target curve position.	Source curve Target curve position

CUT	Cuts out the part of the source curve between Left and Right Limits and stores the result in the target curve position.	Source curve Target curve position Left limit Right-limit
DERIVATE	Differentiates the source curve (first or second order) and stores the result in target curve position. The y-axis of the target curve position will be a normalised scale without unit.	Source curve Target curve position First Order or Second Order
DIV	Divides two curves to gain a third curve which is the quotient of the two curves. The two source curves can have any y-axis unit. The y-axis of the target curve position will be a normalised scale without unit.	First source curve Second source curve Target curve position
HISTOGRAM	Creates a histogram from any non-fraction curve (source curve 1) and a fraction curve (source curve 2_frac), and stores the result in the target curve position. If source curve 2 is not a fraction curve a run time error will occur. The y-axis of the target curve position will be the same as that of the first source curve.	First source curve Second source curve Target curve position
INTEGRATE	Performs a mathematical integration of the source curve and stores the result in Result curve. This instruction is not the same as Peak integrate which performs a real peak integration.	Source curve Target curve position
POOL_ FRACTIONS	Pools fractions from the source curve and stores the result in the target curve position. The fractions are pooled from the first selected fraction to the last selected fraction. If source curve is not a fraction curve, or First or Last is not an existing identification, a run time error will occur.	Source curve Target curve position First fraction to pool Last fraction to pool

RET_MUL	Multiplies the retention of the source curve by the Multiplication factor and stores the result in the target curve position.	Source curve Target curve position Multiplication factor
RET_SHIFT	Shifts the retention of the source curve by the Shift factor and stores the result in the target curve position.	Source curve Target curve position Shift factor
SMOOTH_AR	Smooths source curve with an autoregressive filter and stores the result in target curve position. The Filter parameter decides the strength of the filter.	Source curve Target curve position Filter
SMOOTH_ MA	Smooths the source curve with a moving average filter and stores the result in Resulting Curve. The Filter width parameter decides how many samples wide the filter is.	Source curve Target curve position Filter width
SMOOTH_ MEDIAN	Smooths the source curve with a median filter and stores the result in target curve position. The Filter width parameter decides how many samples wide the filter is.	Source curve Target curve position Filter width
SUB	Subtracts two curves to gain a third curve which is the difference of the two curves. The two source curves must have the same y-axis unit and not be fraction or injection curves.	First source curve Second source curve Target curve position
TDIV	Divides two curves to gain a third curve which is the quotient of the two curves. The two source curves can have any y-axis unit. The threshold values are used to avoid division of numbers close to zero. At those points where source curve 1 has amplitude less than Threshold1, or source curve 2 has amplitude less than Threshold2, the result of the division is defined to be 1.0. The y-axis of the curve will be the same as that of the first source curve.	First source curve Second source curve Target curve position Threshold1 Threshold2

D.4.2 Integration

Instruction	*Description*	*Parameters*
CALCULATE_ BASELINE	Calculates a baseline from the source curve. The baseline is stored in the target curve position. DEFAULT can be selected in the Baseline parameters which will then calculate default baseline parameters for each new curve.	Source curve Target curve position Noise Window Shortest baseline segment Slope limit Max baseline level
CLEAR_ PEAKTABLE	Clears the peak table in Peak table source from the working memory of the computer.	Peak table source
COPY_ PEAKTABLE	Copies a peak table from Peak table source to Resulting peak table.	Peak table source Resulting peak table
NEGATIVE_ PEAKS	Controls the baseline behaviour in subsequent baseline calculations. If OnOff is ON then the baseline may be drawn above the curve and negative peaks may be detected by PEAK_INTEGRATE. If OnOff is OFF then the baseline is never drawn above the curve.	OnOff
PEAK_ INTEGRATE	Performs a peak integration on the source curve and stores the resulting peak table in Resulting peak table. It is assumed that the baseline is subtracted.	Source curve Resulting peak table
PEAK_ WINDOW	Specifies which part of the source curve that will be integrated. Peaks between retention Left limit and Right limit will be detected if the OnOff parameter is set to On. If OnOff is set to Off, the whole curve will be used for integration.	Source curve Left limit Right limit OnOff

REJECT_ PEAKS	Any combination of conditions is allowed. If all parameters are OFF then every detected peak are included in the peak table.	Height less than Width less than Width more than Area less than Peak must be one of xx largest
SET_ COLUMN_ HEIGHT	Sets the column height for the peak integration calculation of the HETP value. The Column height parameter is the height of the column in centimetres. If Column height is OFF then the HETP value is not calculated for the following integrations.	Column Height
SET_ COLUMN_V0	Sets the void volume for peak integration calculation of Kav.	Void volume
SET_ COLUMN_VT	Sets the total liquid volume for peak integration calculation of the capacity factor.	Total liquid volume

D.4.3 File Operations

CURVE_ OPEN	Opens the curve specified in the Result file defined in File name and stores it in target curve position. If "*" is entered as File name the current result file will be used. The File name parameter may include a path from the users root folder.	File name Curve name Target curve position
IMPORT_ CURVE	Imports a curve to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.	Chromatogram name source curve Target curve position
IMPORT_ PEAKTABLE	Imports a peak table to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.	Chromatogram name Peak table source Resulting peak table

PEAKTABLE_ OPEN	Opens the specified Peak table in the Result file defined in File name and stores it in the Resulting peak table. If "*" is entered as File name the current Result file will be used. The File name parameter may include a path from the current users root folder.	File name Peak table name Resulting peak table

D.4.4 Export

Instruction	*Description*	*Parameters*
EXPORT_ CURVE_ ASCII	Exports the Source curve to the file defined in Export to File in ASCII format. In the part of source curve limited by Left limit and Right limit Every <n> samples are exported.	Source curve Left limit Right limit Every <n> sample Export to file
EXPORT_ CURVE_WKS	Exports the source curve to the file defined in Export to File in WKS format. In the part of source curve limited by Left limit and Right limit Every <n> samples are exported.	Source curve Left limit Right limit Every <n> sample Export to file
EXPORT_ EVAL_LOG_ ASCII	Exports an evaluation log in ASCII format to the file defined in Export to file.	Export to file
EXPORT_ EVAL_LOG_ WKS	Exports an evaluation log in WKS format to the file defined in Export to file.	Export to file
EXPORT_ METHOD_ ASCII	Exports a method to the file defined in Export to file in ASCII format. If all parameters are OFF then no method is exported. If Main is ON then the main method is included and if Blocks is ON then all blocks are included in the exported file.	Main Blocks Export to file
EXPORT_ METHOD_ WKS	Exports a method to the file defined in Export to file in WKS format. If all parameters are OFF then no method is exported. If Main is ON then the main method is included and if Blocks is ON then all blocks are included in the exported file.	Main Blocks Export to file

EXPORT_ PEAKTABLE_ ASCII	Exports the peak table in Peak table source to the file defined in Export to file in ASCII format.	Peak table source Export to file
EXPORT_ PEAKTABLE_ WKS	Exports the peak table in Peak table source to the file defined in Export to file in WKS format.	Peak table source Export to file
EXPORT_ DOC_WKS	Exports the documentation in the current result file in WKS format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF RefCurves ONOFF EvalProc ONOFF Method Info ONOFF Method Notes ONOFF StartNotes ONOFF RunNotes ONOFF EvalNotes ONOFF Sys Settings ONOFF Calibration ONOFF LogBook ONOFF Result Name ONOFF Column Parameters NAME Export to file

EXPORT_ DOC_ASCII	Exports the documentation in the current result file in ASCII format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF RefCurves ONOFF EvalProc ONOFF Method Info ONOFF Method Notes ONOFF StartNotes ONOFF RunNotes ONOFF EvalNotes ONOFF Sys Settings ONOFF Calibration ONOFF LogBook ONOFF Result Name ONOFF Column Parameters NAME Export to file

D.4.5 Chromatogram functions

Instruction	*Description*	*Parameters*
COPY_ CHROM	Creates a copy of the specified chromatogram. If "*" is used as source then the current (default) chromatogram is used. If "*" is used as destination then a default name will be created for the copy.	From chromatogram name To chromatogram name
CREATE_ NEW_ CHROM	Creates a new chromatogram with the given name. If "*" is used for the chromatogram name a default name will be generated and used.	Name

DELETE_ CHROM	Deletes the named chromatogram. If trying to delete the current (default) chromatogram a run time error will be caused.	Chromatogram name
OPEN_ CHROM	Opens the specified chromatogram from the specified file.	File name Chromatogram name
RENAME_ CHROM	Renames the specified chromatogram. If "*" is used as From then the current (default) chromatogram is used.	From chromatogram name To chromatogram name
RESTORE_ DESTINATION _CHROM	Resets the destination for the subsequent curve and peak table operations to the default chromatogram. Used in pair with the SET_DESTINATION_CHROM instruction.	
SET_ DESTINATION _CHROM	Opens the named chromatogram as destination for the subsequent curve and peak operations. Used in pair with the RESTORE_DESTINATION _CHROM instruction.	Chromatogram name

D.4.6 Other

BASE	Sets the x-axis base in which the following calculations will be done in. If the value of x-axis base is DEFAULT then the default base is used (usually the base the method was run in). This instruction should be the first in the evaluation procedure otherwise it will have no effect at all.	X-axis base
COMMENT	Inserts a comment below the marked instruction	Comment text
ENDLOOP	Marks the end of a LOOP statement.	

LOOP	The instructions between this statement and the ENDLOOP statement are repeated n times. It is possible to have loops within loops as long as the number of LOOP statements matches the number of ENDLOOP statements.	n Number of loops
REPORT	Prints a report with the specified named report layout and title. If Title is "*" then the title in the report layout is used. If ReportLayout is "*" then a default layout is used.	Report layout Report title
RUN_ PROGRAM	Starts a program as a separate process. The Program name string contains the program name and parameters to start it with.	Program name

E Feedback tuning

Some systems allow you to perform PID feedback tuning of the pump flow rate using the **System:Tune** command in System control. Other systems allow you to perform tuning with the insertion of a tune instruction in the method. Other systems such as the ÄKTAdesign systems do not use tuning at all.

This appendix describes the principles of PID feedback tuning of pump flow rate to available systems. UNICORN process control software provides a graphical interface for feedback tuning.

Feedback control is aimed at eliminating discrepancies between the actual value and the requested value (in this case flow rate). BioProcess System uses the measured flow rate to control overall pump speed and the measured conductivity to control the relative speeds of pumps A and B in gradient formation.

PID tuning uses three parameters to determine feedback control:

- The P parameter reduces the effect of an error but does not completely eliminate it. A simple P-regulator results in a stable discrepancy between actual and requested flow rate (*a stationary error*).
- The I parameter eliminates the stationary error, but results in a slight instability leading to oscillations in the actual flow. I can have values between 0 and infinity, where smaller values have greater effect and a value of infinity has no effect. (The value infinity is set as 9999 in UNICORN).
- In certain cases, the D parameter can reduce the oscillations introduced by a PI-regulator. D can have values between 0 and infinity, where larger values have greater effect and a value of 0 has no effect. Most often, a simple PI-regulator is preferable for control of flow rate, and BioProcess System is configured by default with the D parameter set to zero.

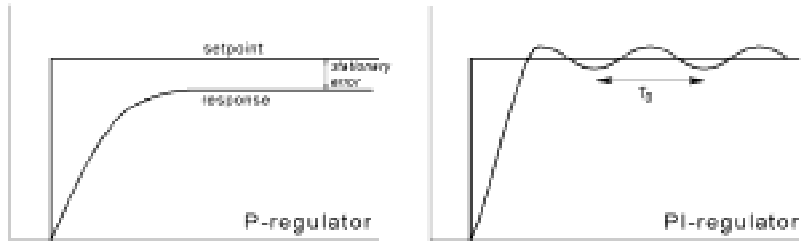


Figure E-1. A simple P-regulator (left) gives a stationary error. A PI-regulator (right) eliminates the stationary error but introduces stable oscillations in the response.

Tuning a feedback control system in practice is largely a matter of trial and error. The following recommendations summarize the Ziegler-Nichols method for finding suitable PID-values (small empirical adjustments in the suggested values may be required for optimal feedback control).

E.1 Flow rate tuning

1. Make sure that the flow path is open.

   Select **System:Tune** in System control. Select **Flow for Feedback Control**. Open the valves and start the pump with the column in-line.

2. Set P=0.05, I=9999 and D=0. Set the flow rate in **Setpoint**. Press **New parameters** and the new values will apply.

3. Note the response. Increase the value of P until the actual flow rate oscillates with a constant period and amplitude.

Note: When changing to new PID values, set the new values and flow rate and press **New parameters**. The flow rate must be changed in **Setpoint** every time the PID values are changed.

When the oscillation is satisfactory, note the P value (P0) and the oscillation period in seconds (T0).

4. Calculate suggested PID values for the required regulator type from the table below.

Regulator type	P	I	D
P	0.5 * P_0		
PI	0.45 * P_0	0.83 * T_0	
PID	0.6 * P_0	0.5 * T_0	0.125 * T_0

5. Adjust the PID parameters from these suggested starting values until the feedback behaviour is satisfactory.

6. When satisfied, press **Save** to save the PID parameter settings.

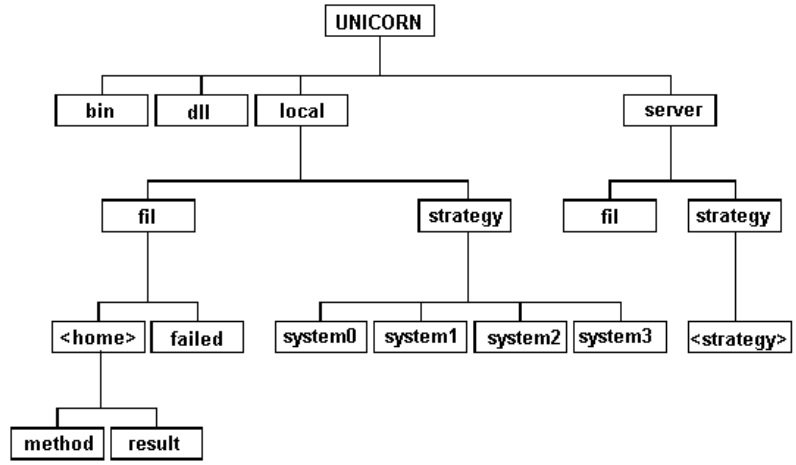
During tuning adjustments, UNICORN displays the effects of the current parameters graphically. The **Output** signal is the signal to the pump. The **Input** signal is the actual flow rate.

E.2 Gradient tuning

Tune gradient settings in the same way as flow rate settings, with the exception that the **Setpoint** parameter is %B. The **Input** signal is the actual eluent concentration (%B) as determined by the conductivity.

F File organisation

This appendix documents the file structure in a UNICORN installation.



F.1 Stand-alone installations

In a stand-alone installation, the entire folder structure resides on the local hard disk. System and user definition files and system strategies are duplicated in the local and server folders.

F.2 Network installations

F.2.1 Local and remote computers

The bin, dll and local folder structures are installed on each computer.

F.2.2 Network server

The server folder structure is installed on the network server. The local\fil folder structure is also installed if home folders are created on the server disk.

System and user definition files and system strategies are copied automatically from the network server to each station, so that local stations can be used as stand-alone systems in the event of a network communication failure.

folder	*Description*
UNICORN	The main UNICORN folder.
– bin	Executable modules.
– dll	Device drivers and dynamic linked library (dll) modules. the drivers are installed in the NT system folder for device drivers: \winnt\system32\drivers
– local	Holds a copy of the global system definitions (system.tab). Not available on remote computers.
– – fil	Holds a copy of the files containing user definitions (user30.mpm), Column list (columns.cmn) and BufferPrep recipes (global.rcp, comp.rcp). Not available on remote computers. Global audit trail files are also stored here.
– – – <home>	Home folders for users in the system. There may be any number of home folders in the system. In network installations, home folders may be created on the server disk as well as on local disks.
– – – – method	Holds method files, MethodQueue folders and user-created method folders.
– – – – result	Holds result files, scouting result folders and user-created result folders.
– – – failed	Holds result files in the event of network communication failure.
– – strategy	Holds sub-folders for each system physically connected to the local computer.
– – – system0 – – – system1 – – – system2 – – – system3	System information for systems physically connected to the local computer. Holds a copy of the strategy, flow scheme and templates, and files for the system settings and system audit trail. folder system0 corresponds to installed System 1. Folders for systems that are not installed are empty.
– server	Holds the original file for system definitions (system.tab). This file is copied to the local folder.

– – fil	Holds the original files for user definitions (users30.mpm), global procedures (globproc.gpl), global report formats (globproc.grf), BufferPrep recipes (global.rcp, comp.rcp) and the column list (columns.cmn). The user definition file, Column list and BufferPrep recipes are copied to the local\fil folder.
– – strategy	Holds the folder structure for installed strategies.
– – – <strategy>	Holds the original files for installed strategies. There is one <strategy> folder for each installed strategy. Each <strategy> folder also holds the corresponding template methods (if installed). Strategy files are copied to the local\strategy folder according to the systems installed on the local station.

Index

A

About UNICORN button 3-3
access 14-11
access level
    examples 14-6
access level authorisation items 14-4
access levels
    defining 14-4
access security 11-1
ActionIfPCFail setting 15-4
Activity histogram 9-32
adding blocks 5-9
adding curves 5-38, 9-17
Adjust retention zero to injection number 9-9
administration
    overview 14-1
    user 14-9
adviser
    on-line 2-4
alarms
    display 15-2
    introduction 15-2
alarms and monitors instructions B-6, C-5
alarms and monitors manual instructions B-9
alarms and non manual control instructions C-9
alarms and warnings 6-16
alarms system settings instructions B-10, C-10
algorithm
    autoregressive D-1
    median D-2
    moving average D-1
    smoothing D-1
All with this unit 9-7
amplitude multiply 9-30
Analysis module (optional) 1-2
answers
    input field 5-29
    multiple choice 5-29
    no answer 5-30
    value 5-30
Apply to all chromatograms 9-4
ASCII export format 10-37
asymmetry D-9
asymmetry, measuring in peak 10-20

audit trail
    backing up audit trail files 14-16
    renewing audit trail files 14-16
audit trail/system setup 14-4
audit trails 14-13
    examining 14-13
    global 14-13
    printing 14-16
    system 14-14
    viewing older audit trails 14-15
authorisation items 14-4
authorisation profile example
    development staff 14-7
    process operators 14-8
    process supervisors 14-8
    system administrator 14-6
automated evaluation procedures 10-28
Autoregressive 9-14
autoregressive algorithm D-1
autosave interval value 14-3
Averaging time setting 15-5
axes
    fixed scale 9-12
Axis Scale, Fixed 9-9

B

backup security 3-13
base
    changing 5-21, 5-33
    choosing 5-10
    instruction 5-49
    type 5-21
baseline
    calculation theory D-2
    default parameters values 10-6
    deleting baseline data points 10-15
    drawing the baseline D-5
    drawing the new baseline 10-15
    estimating the baseline parameters from the source curve D-5
    finding baseline segments D-3
    insertion of baseline data points 10-14
    manually editing 10-13
    measuring noise level using curve co-ordinates D-5
    measuring shortest baseline segment using curve co-ordinates D-5
    measuring slope limit using differentiate and curve co-ordinates D-6
    optimising the baseline parameters 10-6
    selecting baseline points D-5
baseline calculation 10-1

baseline problem
    baseline on top of peaks 10-12
    baseline slope does not follow the source curve 10-7
batch runs 10-33
BioPilot
    calibrating monitors 6-27
BioPilot System, sampling frequency A-3
BioPilot, methodbase instruction 5-51
BioProcess System, calibrating monitors 6-28
block
    adding 5-9
    calling 5-8
    calling from an existing block 5-11
    choosing base 5-10
    Clean after gradient 4-6
    Column equilibration 4-5
    controlling block and method length 5-52
    deleting 5-11
    entering length 5-10
    Fractionation 4-5
    Gradient 4-6
    Load sample loop 4-5
    Main 4-5
    naming 5-10
    renaming 5-12
    restoring deleted block 5-12
    Sample injection 4-5
    Start condition 4-5
    strategy for creating 5-11
    viewing 5-7
    Wash out unbound sample 4-5
    window 5-7
blocks
    copying 5-13
    importing 5-13
    in method templates 4-4
blocks and instructions, relationship 2-6
blocks in methods 2-5
blue square beside text 5-15
bold text 5-15
breakpoint
    changing 5-18
    moving instruction within breakpoint 5-20
breakpoints 2-6
breakpoints during gradients 5-56
buffer
    capacity A-3
buffer, defining a new salt 5-75
buffer, defining a new substance 5-74

BufferPrep 2-8, 5-40
creating a recipe 5-72
display in start protocol 6-4
display of recipe at start of run 5-46
editing a recipe 5-76
fine tuning recipe with correction factors 5-76
BufferPrep, display of recipe at start of run 5-46

C

Calculate baseline 10-1
calculating a baseline 9-17
calibrating monitors 6-22
calibrating monitors for ÄKTAdesign systems 6-23
calibration data display 6-5
calibration, display of calibration settings at start of run 5-47
calling block 5-11
calling blocks 5-8
calls, unconditional and conditional 5-9
capacity factor D-9
changing 14-11
chromatogram
comparing chromatograms from different runs 9-19
contents 9-2
copying curves into one chromatogram 9-26
displaying a grid 9-10
entering text 9-17
importing chromatograms to compare 9-20
optimising presentation 9-2
optimising the workspace 9-3
printing active chromatograms 9-32
renaming 9-19
temporary 9-2
viewing the curves 9-3
window adjustments 9-2
Chromatogram Layout
specifying report layout changes 9-36
chromatogram layout
changing curve names 9-5
choosing curves displayed 9-5
editing 9-4
opening the chromatogram layout dialogue 9-3
chromatographic technique selection 5-2
chromatographic techniques available 4-2
chromatography systems used with Unicorn i, 1-1
Clean after gradient 4-6
Close button 6-16
colour
changing colour of curve 9-6

changing colour of text and background in run data window 6-8
changing curve colour 6-9
column
adding a new column 5-67
deleting 5-71
display selected columns in start protocol 6-4
editing parameters 5-71
list 5-66
parameter, any 5-50
parameter, named column 5-50
parameters 5-39
selecting or changing in a method 5-71
Column equilibration 4-5
column, choosing 4-3
columns
display of definitions at start of run 5-46
communication failure 6-17
comparing chromatograms from different runs 9-19
comparing curves 9-22
comparing runs 9-19
computer
specifications A-1
concentration and gradient, eluent 5-55
Cond 10-27
Cond% 10-27
conditional (Watch) instructions 5-8
conditional calls 5-9
conditional instructions 5-58
CondRefTemp setting 15-6
CondTempComp setting 15-6
conductivity cell
entering new cell constant 6-26
conductivity cell constant, calibration 6-26
connection
control and view mode connections 2-10
system 2-10
connection modes 6-18
connection security 11-1
connection, establishing 6-18
control
capacity A-2
facilities 2-9
manual 6-13
software modules 2-3
control mode connections 2-10
control unit number selection 14-3
Controlled by 6-15
copying blocks 5-13
copying files and folders 3-9

copying files from external 3-10
copying files to external 3-9
Correlated baseline 10-1
creating user groups and users 12-9
cursor
    vertical 5-32, 6-9
cursor line, vertical 5-20
curve
    adding curves 9-17
    calculating a baseline 9-17
    changing and fixing the axes 9-7
    changing colours and styles 6-9
    changing curve names 9-5
    changing the colour and style 9-6
    comparing curves 9-22
    components of a curve name 9-5
    copying curves into one chromatogram 9-26
    creating 10-25
    cut 9-12
    export 10-36
    importing a blank run curve 9-16
    importing curves 9-22
    importing individual curves 9-25
    matching protein activity to a curve 9-32
    mirror 9-25
    move 9-29
    moving a curve using the Shift function 9-30
    normalise 9-29
    overlay 9-25
    renaming 9-19
    selecting curves displayed on screen 6-8
    showing part of a curve 9-11
    size 9-29
    smoothing 9-13
    stack 9-25
    stacking and stretching curves 9-27
    stacking and stretching curves using the normalise function 9-28
    stretching and shrinking a curve using multiply 9-30
    subtracting a blank run curve 9-15
    subtracting the blank run curve 9-17
curve comparisons 2-10
curve manipulation 2-10
curve settings 15-7
curve system settings instructions B-14
curves
    adding 5-38
    deleting 5-39
    renaming 5-39
curves system settings instructions C-12

cut curve 9-12

D

data
    buffers A-2
    buffers capacity A-3
    export 10-36
    minimising loss in the event of communication failure A-2
    transfer A-2
data security 11-2
default parameters in methods 2-6
delete
    instruction 5-17
Delete button 6-16
deleting
    blocks 5-11
    files or folders 3-12
deleting text in a chrmoatogram 9-18
Delta_Base setting 5-60
Delta_Peak setting 5-59
Demo system 13-10
dialogue
    Batch run 10-33
    BufferPrep recipes 5-72
    calibration constant 6-26
    Chromatogram Layout, Curve tab 9-4
    Chromatogram Layout, Y-axis tab 9-8
    Column List 5-67
    Condition 8-3
    conductivity cell constant 6-26
    Copy from external 3-11
    Copy to external 3-10
    Create Curve 10-25
    Create new unit 10-26
    Curve Name 9-6
    Curve Properties, Curve Style and Colour tab 6-10
    Curve Properties, Curves tab 6-9
    Curve Properties, X-Axis tab 6-11
    Curve Properties, Y-axis tab 6-10
    Data Run Properties, Run Data Layouts tab 6-7
    Delete blocks 5-12
    Delete Folder 14-13
    Differentiate 10-24
    Divide 10-22
    Edit column 5-67
    Enter Information 13-16
    Export Curve 10-37
    Export Documentation 10-36

Filter 3-7
Find file 3-8
Generate Report 9-34
Import Block 5-13
Import procedures 5-36
Import Reference Curve 5-38
Instant Run 3-3
Instructions, Alarms group 15-1
Integrate 10-2
Leave Control 6-20
Line Style and Colour 9-7
Load MethodQueue 8-2
Manual instruction 6-16
MethodQueue Editing 8-2
MethodQueue Editor 8-1
MethodQueue Setup 8-3
Method-System connection 3-11
Multiply 9-31
New block 5-10
New method 4-2, 5-2
New system 14-2
Open curves 9-26
Open Log File 14-16
Password and System Control Window Settings 13-12
pH calibration 6-24
Print 9-33
Print Options 9-33
Procedure editor 10-29
Procedure editor, Editing procedures 10-30
Procedure editor, instructions field 10-31
Reject Peaks 10-5
Rename Blocks 5-12
Saving a method 4-7
Scouting Variables 5-26
Select Components 13-9
Select Drive 13-10
Setup Type 13-11
Smooth 9-14
Stack Offset 9-25
Start Copying Files 13-13
Strategy & Template Installation 13-15
System connect 6-18
System Installation 13-14
System Setup 13-14
temperature calibration in the conductivity cell 6-24
Toolbar Guide 2-2, 3-2
Unicorn Setup 13-18
Unicorn Software License Agreement 13-8
User information 13-8

User set-up 14-9
Variable name definition 5-23
Welcome 13-7
Windows 5-6
Divide 10-21
Draw spline 10-27
Draw straight to next point 10-27

E

edit
BufferPrep recipe 5-76
chromatogram layout 9-4
column parameters 5-71
editing text inserted in a chrmoatogram 9-18
existing procedure 10-30
method instructions 5-5
method variables 4-4
MethodQueues 8-4
methods, overview 5-1
parameters in procedure instruction 10-31
procedures 5-37
system definitions 14-3
text instructions 4-10, 5-5
eluent concentrations and gradients 5-55
eluent, setting the initial eluent composition 5-56
evaluating results 10-1
Evaluation
exit 9-40
functions and instructions D-1
evaluation
overview of evaluation facilities 2-10
Evaluation button 3-4
evaluation module
overview 2-3
evaluation procedure
editing 10-30
evaluation procedures 5-34
chromatogram functions D-17
curve operations D-10
display after run 6-4
export D-15
file operations D-14
integration D-13
miscellaneous D-18
potential problems 10-32
reporting 10-35
running 10-33
evaluation procedures, automated 10-28

evaluation procedures, display at start of run 5-46
Execute button 6-16
exiting Evaluation 9-40
export
    data or curves 10-36
    results 10-36

F

failed folder access 11-2
feedback control, aims E-1
feedback tuning 6-28, E-1
file
    copying from external 3-10
    copying to external 3-9
    deleting 3-12
    file organisation F-1
    file organisation in local and remote computers F-1
    file organisation in network installations F-1
    file organisation in network server F-1
    file organisation in stand-alone installations F-1
    filtering files displayed 3-6
    finding 3-7
    moving and copying 3-9
    presenting 3-5
    renaming 3-12
    sorting order 3-6
files
    method files overview 2-4
    opening and running 3-4
    result files overview 2-4
filter files displayed 3-6
Filter type 9-14
filtering peaks from view 10-4
finding files 3-7
Fixed y-axis 9-7
flow rate tuning E-2
flow scheme display 6-12
flow scheme window 5-21
flowpath
    instructions B-3, C-2
    manual control instructions C-9
    manual instructions B-9
folder
    creating a new folder 3-4
    defining new home folder 14-12
    deleting 3-12
    moving and copying 3-9
    renaming 3-12

user access 14-11
Frac- Parameters setting 15-4
fraction histogram 2-10
Fractionation 4-5
fractionation type identification letter 4-3
FractionNumbering Mode setting 15-4
framework in method 2-6

G

generate report 14-21
generating report from the main menu 14-18
ghost peaks removal 9-13
global audit trails 14-13
Gradient 4-6
gradient
display in start protocol 6-4
forming a step gradient 5-55
tuning E-3
gradient lengths, changing 5-19
gradient page 5-31
gradient profile graphical view 4-6
gradient window 5-8, 5-20
gradient, display at start of run 5-46
gradients
with variable duration 5-57
graphical view of the gradient profile 4-6
grid in chromatogram window 9-10

H

hardware
requirements A-1
hardware installation 13-2
hatch marks 5-33
hatch marks, viewing 5-21, 6-12
help
on-line 2-3
HETP D-10
calculation 10-20
measurement 10-19
home folder
deleting 14-12
new 14-12
home folder selection 14-11
hysteresis setting 15-3

I

import
    chromatograms to compare 9-20
    procedures 5-36
importing a blank run curve 9-16
importing blocks 5-13
Insert button 6-16
installation
    address setting 13-3
    hardware 13-2
    operations required after installation 14-1
    selecting the components 13-9
    selecting the type of system 13-15
    setting IRQ and address 13-3
    stand-alone A-2
    Unicorn software 13-6
installation as stand-alone or in network 2-1
installation of Unicorn on network 12-3
installing
    program files after the initial installation 13-19
    software components after the initial installation 13-19
    strategy files after the initial installation 13-20
    system after the initial installation 13-19
    template files after the initial installation 13-20
    user information after the initial installation 13-19
installing IPX/SPX on the computer 12-10
installing TCP/IP on the computer 12-5
installing Unicorn 12-11
installing Unicorn, NT domain with TCP/IP 12-8
Instant Run button 3-3, 6-3
instruction
    changing 5-18
    deleting 5-17
    insert 5-17
    moving within a breakpoint 5-20
instructions
    adding 5-16
    alarms and monitors B-6, C-5
    at the same breakpoint 5-52
    conditional 5-58
    flowpath B-3, C-2
    for systems using the Unicorn controller box C-1
    manual 6-15
    manual control B-9
    miscellaneous B-8, C-7
    pump B-1, C-1
    system setting B-10
    viewing 5-14

watch 5-8, B-7, C-6
instructions groups 15-2
instructions in methods 2-5
instructions manual
alarms and mon C-9
alarms and monitors B-9
flowpath B-9, C-9
miscellaneous B-10, C-9
pump C-8
instructions system settings
alarms B-10, C-10
curve B-14
curves C-12
monitors B-12, C-12
specials B-11, C-11
instructions, conditional 5-8
Integrate_and_Print 5-34
integrating peaks 10-1
integration D-13
common problems 10-7
including negative peaks 10-6
optimising 10-4
performing 10-2
integration problem
noise detected as peaks 10-10
peak limits too high up on the peaks 10-8
peaks missing 10-11
introduction to Unicorn 1-1
IPX/SPX, installing on the computer 12-10
IRQ setting 13-3

K

Keyboard setting 15-4
Keys setting 15-7

L

layout
saving and applying 9-10
length, entering for a block 5-10
license agreement 13-8
linear flow rates 5-55
Load sample loop 4-5
local station 2-12, 12-2
local station failure 11-3
Locked 6-20
Locked by 6-15
logbook 6-13

logging off 3-13
logging on 3-1
Logoff button 3-13
Logon/Logoff button 3-3
loop symbol with text 5-15

M

Main 4-5
Main menu
    for starting a method 6-2
main menu
    functions overview 2-3
managing system connections 6-17
manual
    assumed skills of user ii
    bold typeface entries ii
    contents overview i
manual control 6-13
manual control instructions B-9
manual instructions 6-15
Manual menu 6-15
Max baseline level adjustment 10-13
maximising resolution A-3
Median 9-14
median algorithm D-2
menu
    manual 6-15
messages during run 5-53
method
    base 2-5
    blocks 5-7
    controlling block and method length 5-52
    creating a new method 4-1
    creating from method templates 4-1
    creating new method 5-2
    default notes in template 5-34
    developing from scratch 5-2
    display of instructions at start of run 5-45
    display of method information at start of run 5-47
    display of notes at start of run 5-46
    editing operations summary 5-6
    editing the variables 4-4
    editor 5-2
    editor modes 5-4
    execution in MethodQueues 8-5
    framework 2-6
    in automatic method scouting 2-6
    information 5-42

instructions 2-5
instructions entering, editing and deleting 5-14
naming 4-8
notes 4-6
notes entry 5-34
open file 3-4
overview creating and editing 5-1
pausing 5-54
preparing for scouting 5-26
running file 3-5
saving 4-7, 5-48
saving method as a template 5-49
starting a method 6-2
starting a method from the Main menu 6-2
starting a method from the System control 6-2
templates 2-7
text instructions display in start protocol 6-4
using selected method instructions 5-49
variables 2-6, 5-22
viewing notes 5-33
method editor
overview 2-3
method editor interface 5-4
method file
copying from external 3-10
copying to external 3-9
method files overview 2-4
method information
display 6-4
method instructions
for systems using Unicorn controller box C-1
pump B-1
methodbase instruction 5-51
Methodbase setting 15-4
MethodQueue
adding more method steps 8-2
Condition command 8-3
defining 8-1
displaying 8-6
editing 8-4
End 8-6
execution of methods 8-5
Exit 8-6
folders and icons 8-4
Immediate start command 8-3
Restart 8-6
running 8-5
selecting the method 8-2
setting a time interval for starting a selected step 8-3

setting up 8-1
Start at 8-6
Start time command 8-3
MethodQueue facilities 2-9
MethodQueues
overview 8-1
methods
concepts and principles 2-4
migrating from UNICORN 2.30 to UNICORN 3.00 13-1
mirror curves 9-25
modes
connection 6-18
monitors
calibrating 6-22
calibrating for ÄKTAdesign systems 6-23
monitors system settings instructions B-12, C-12
Moving average 9-14
moving average algorithm D-1
moving files and folders 3-9
multiply 9-30
multiply, retention or amplitude 9-30

N

names of templates 4-3
naming method 4-8
network
communication error 12-2
communication failure security 11-2
computer installations A-2
considerations 2-11, 6-21
controlling systems from local and remote stations 12-2
creating home folder 14-10
data transfer A-2
example 2-11
installation 3-2
installation of Unicorn 12-3
maintenance 12-1
minimum requirements for running UNICORN 12-3
requirements A-2
security implications 12-1
setup 12-1
setup introduction 12-1
sharing a folder, NT domain with TCP/IP 12-5
sharing a folder, NT workstations connected to a Novell server 12-9
supported network cards A-2
network installation 2-2
network, creating user groups and users, NT domain with TCP/IP 12-4
network, creating user groups and users, NT workstations connected to a

Novell server 12-9
New Method button 3-3
new method creation 4-1
new method, creating 5-2
New unit button 10-25
No Template 5-2
Noise window adjustment 10-10, 10-11
noise,reducing 9-13
normalise function 9-28
notes 4-6, 5-33
display in start protocol 6-4
entering 5-34
in run documentation 9-38

O

on-line adviser 2-4
on-line help 2-3
open
chromatogram layout dialogue 9-3
mehod file 3-4
result file 9-1
OutletValve setting 15-5
overlay curves 9-25

P

parameter setting
changing 15-2
restoring default value 15-2
parameter settings, viewing 15-2
password 14-11
password entry 3-1, 14-10
passwords 13-11
peak
deleting a peak in the peak table 10-16
identification 10-21
integration 2-10
joining a peak 10-16
measuring peak asymmetry 10-20
peak table column components D-6
purity 10-21
purity and identification 2-10
splitting a peak 10-16
peak table
renaming 9-19
Peak_Frac- Parameters setting 15-4
peaks
adjusting peak start and end points 10-17

adjusting the peak limits 10-15
changing peak labels 10-4
direct measurement of retention time and peak heights 10-18
excluding peaks from integration 10-5
filtering peaks from view 10-4
identification names for peaks 10-18
integrating 10-1
measuring retention time and peak heights 10-18
optimising peak integration 10-4
reject peaks 10-5
Permissions button 12-5
pH, calibrating the pH sensor 6-24
pH, fine tuning with correction factors 5-41
pHCell setting 15-6
pHTempComp setting 15-6
PID
feedback tuning E-1
tuning parameters E-1
Point list 10-14
Point mode button 10-14
pooling fractions 9-18
pressure limit adjustment 4-4, 5-4
pressure, setting reading to zero 6-23
print
active chromatograms 9-32
audit trails 14-16
printer setup 3-13
printing scouting results 7-6
reports 9-33
Print_Chromatogram 5-34
procedure
placing on the menu and running 10-35
recording 10-28
removing 10-32
renaming 10-32
procedure instructions
editing the parameters 10-31
inserting new instructions 10-31
removing instructions 10-31
saving the edited procedure 10-31
viewing the parameters 10-30
procedures
defining and viewing 5-35
deleting 5-36
editing 5-37
importing 5-36
renaming 5-37
selecting to run 5-36
protein, matching protein activity to a curve 9-32

pump instructions B-1, C-1
pump manual control instructions C-8
PumpGain setting 15-5
PumpType setting 15-4

Q

questions
    defining for answer type 5-29
    deleting 5-31
    display at start of run 5-45
    display in start protocol 6-4
    editing 5-31
    Input field 5-29
    inserting 5-31
    multiple choice 5-29
    no answer 5-30
    types mandatory, authorised, chromatogram 5-28
    value 5-30
quitting Unicorn 3-13

R

recording a procedure 10-28
red bullet beside text 5-15
reference curve
    display in start protocol 6-4
reference curves 5-37
reference curves, display at start of run 5-46
reject peaks 10-5
remote station 2-12, 12-2
renaming
    files or folders 3-12
report
    generate 14-21
    generate from System Control 14-22
    generating from the main menu 14-18
    preview 9-34
    printing report 9-33
    saving a report format 9-36
    selecting a format 9-36
    specifying chromatogram in report 9-35
report generator
    ÄKTA hardware information 14-20
    attachments 14-20
    integrity check 14-21
    problem description 14-19
    reproducibility information 14-19
    systems list 14-18

report Generator Wizard 14-17
reports
    overview 2-11
resolution D-9
    maximising A-3
resolution and sampling frequency A-3
restoring deleted block 5-12
result
    changing result name after start of run 5-47
    opening a result file 9-1
    presenting results, overview 9-1
    saving results 9-32
    viewing scouting results 7-6
result file
    contents 9-2
    copying from external 3-10
    copying to external 3-9
    specifying name 6-5
result files
    overview 2-4
result name 5-43
results
    after peak integration 10-3
    copying results to the clipboard 10-37
    export 10-36
retention
    direct measurement of retention time and peak heights 10-18
    measuring retention time and peak heights 10-18
retention multiply 9-30
run
    changing scouting settings during a run 7-5
    comparing different runs 9-19
    comparing scouting runs 7-6
    control mode connection 4-9
    data 6-6
    documentation 9-37
    editing method variable values 4-9
    mehod file 3-5
    messages during run 5-53
    MethodQueue 8-5
    monitoring 6-5
    performing 6-1
    start protocol pages 4-9
    starting 4-8
    starting an instant run 6-3
    viewing information about a run 9-9
run data 6-6
run data layout 6-7
run data window style 6-8

run documentation
calibration 9-39
evaluation log 9-40
logbook 9-39
notes 9-38
scouting 9-37
variables 9-37
Run setup 5-6
run setup 5-25
Run/Excluded button 7-5

S

salt concentrations, measuring in the fractions 10-27
sample application type identification letters 4-3
Sample injection 4-5
sampling frequency A-3
save
edited procedure 10-31
layout 9-10
method 4-7, 5-48
method as a template 5-49
procedures 10-29
report format 9-36
scale, changing scale of x-axis 6-11
scale, changing scale of y-axis 6-10
scouting
changing scouting settings during a run 7-5
choosing the variables 7-2
comparing runs 7-6
continuous gradients scouting example 7-7
defining runs in scheme 5-27
deleting and inserting run columns 7-2
deleting columns 5-28
description 2-8
display of scheme at start of run 5-45
displaying start protocols in scouting run 5-47
examples 7-7
in run documentation 9-37
inserting scouting runs before an existing run 5-28
overview 7-1
performing a run 7-5
pH scouting example 7-8
preparing a method 5-26
printing results 7-6
results 7-5
sample volume example 7-7
scheme set-up 7-4
screening different columns example 7-7

setting contents of report print 7-6
setting parameters 5-26
setting up 7-1
setting variables in the scouting page 7-4
start protocol 5-28
start protocol settings 7-4
variables 6-4
viewing results 7-6
security
access 11-1
backup 3-13
connection 11-1
data 11-2
in network 12-1
recommendations for control stations 11-3
security features 2-13
security features, overview 11-1
security system in Unicorn 1-2
SetCondScale setting 15-5
SetCondScale0% 15-5
SetCondScale100% 15-5
setting
ActionIfPCFail 15-4
Averaging time 15-5
CondRefTemp 15-6
CondTempComp 15-6
Frac- Parameters 15-4
FractionNumbering Mode 15-4
hysteresis 15-3
Keyboard 15-4
Keys 15-7
Methodbase 15-4
OutletValve 15-5
Peak_Frac- Parameters 15-4
pHCell 15-6
pHTempComp 15-6
PumpGain 15-5
PumpType 15-4
SetCondScale 15-5
Store 15-7
Time between samples 15-7
WatchPar 15-6
Wavelength 15-5
settings
curve 15-7
display at start of run 5-47
in monitors group 15-5
monitor settings 15-5
specials group 15-4

shortcut keys iii
Shortest baseline segment 10-6
Shortest baseline segment adjustment 10-13
sigma D-8
Slope limit 10-6
Slope limit adjustment 10-9
slope values 10-23
Smooth 10-22
smoothing a curve 9-13
smoothing algorithms D-1
smoothing parameter 9-14
software requirements A-1
sorting order of files 3-6
specials system settings instructions B-11, C-11
Spline through 10-27
stack curves 9-25
Stack Offset 9-25
stacking and stretching curves 9-27
stand-alone installation 2-1, A-2
start
    Unicorn 3-1
Start condition 4-5
start protocol 5-44, 6-3
starting a run 4-8
station
    remote and local 2-12
status bar text messages 6-15
step gradient 5-55
Store setting 15-7
strategies
    what are strategies 2-1
strategy
    finding description of instruction B-1
    for ÄKTAexplorer 100 B-1
    selection 14-3
strategy for creating blocks 5-11
Subtract 10-21
subtracting a blank run curve 9-15
subtracting the blank run curve 9-17
system
    connection 6-18
    connection modes 6-18
    connections management 6-17
    disconnect 6-19
    disconnecting 6-20
    leaving and locking 6-20
    naming 14-3
    requirements A-1
System # button 8-1

system audit trails 14-14
System Control button 3-4
system control module
    overview 2-3
system definitions
    defining new system 14-2
    deleting 14-3
    editing 14-3
    management 14-2
System is available 6-15
system run hours 14-17
system settings
    accessing settings 15-1
    instructions B-10
    introduction 15-1
system settings display 6-5

T

TCP/IP, installing on computer 12-5
technique identification letters 4-3
technique selection 5-2
techniques available using chromatography 4-2
temperature, calibrating the temperature sensor 6-24
template
    deleting 5-49
    identification letters 5-3
    VarpH 4-3
    viewing information 5-3
template names 4-3
templates
    creating methods from method templates 4-1
    method 2-7
temporary chromatogram 9-2
temporary chromatogram, clearing contents 9-2
text entry in chromatogram 9-17
text instruction window 5-8
text instructions editing 4-10
text instructions editor 5-5
threshold values 10-21
Time between samples setting 15-7
tool bar in system control workspace 6-13
toolbar
    buttons 3-3
    method editor 5-4
toolbar buttons 2-2
toolbar guide 3-2
typographical conventions ii

U

unconditional calls 5-9
undo zoom 5-21
Unicorn
    chromatography systems i, 1-1
    concepts 2-1
    installation 12-11
    installation summary 13-1
    installing in NT domain with TCP/IP 12-8
    installing on network with IPX/SPX - Novell server 12-8
    installing software 13-6
    introduction 1-1
    main menu windows 3-4
    network installation 3-2
    operations after installation 14-1
    optional Analysis module 1-2
    quitting 3-13
    security features 2-13
    security system 1-2
    starting 3-1
    summary of functional features 1-1
    system administrator 12-1
    user interface 2-2
UNICORN, migrating from an earlier version 13-1
Unlocked 6-20
user
    creating a new home folder 14-10
    defining new user 14-10
    deleting 14-12
    home folder selection 14-11
    identification 14-9
    password 14-10
    selecting user accessible folders 14-11
    viewing and changing user definitions 14-12
user administration 14-9
user groups and users, creating on local computer 12-9
user groups and users, creating on NT domain with TCP/IP network 12-4
user groups and users, creating on NT workstations connected to a Novell server 12-9
user interface 2-2
username entry 3-1

V

Var button 5-22
variable
    removing 5-24
    renaming 5-24

variables 5-45
    defining 5-23
    editing in run setup 5-25
    for scouting 6-4
    identifying 5-22
    in run documentation 9-37
    in run setup 5-25
    method 5-22
    start protocol items 6-3
variables in methods 2-6
VarpH template 4-3, 5-4
vertical cursor line 5-20, 5-32, 6-9
view mode 3-5
View Windows button 6-5
viewing blocks 5-7
viewl mode connections 2-10

W

Wash out unbound sample 4-5
watch
    instructions B-7, C-6
watch actions 5-61
watch conditions 5-58
watch conditions settings 5-59
watch examples 5-62
watch instructions 5-8
WatchPar setting 15-6
Wavelength setting 15-5
window
    Baseline box with Shortest baseline segment and Noise D-4
    Create Curve chromatogram 10-26
    curves 6-8
    Cut 9-12
    Edit Baseline chromatogram 10-14
    Edit Peak Table chromatogram 10-16
    flow scheme 5-21
    general window techniques 6-6
    hide 6-6
    Method 3-5
    Method editor flow scheme 5-21
    Method editor gradient 5-20, 5-32
    Normalise 9-29
    Peak Window chromatogram 10-5
    text instruction 5-14
Windows NT A-1
wizard, report generator 14-17
WKS export format 10-37

X

X-Axis adjustment 9-8
x-axis, changing scale 6-11

Y

Y-Axis adjustment 9-7
y-axis, changing scale 6-10

Z

Zero baseline 10-1
Ziegler-Nichols method E-2
zoom
    undo 5-21
zoom function 5-21, 6-12
zoom function in chromatogram window 9-11
zoom function in gradient page 5-32



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